Towards a better understanding of serous carcinoma of the female genital tract

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CONTENT

Chapter 1

General introduction

Endometrial cancer is the most common gynecologic malignancy in the western world and the fourth commonest site after breast, colorectal, and lung cancer.[1, 2] It accounts for 1,930 new cases and 425 cancer-related deaths in the Netherlands each year.[3] Most cases are diagnosed after menopause, with the highest incidence around the sixth decade of life.[4] Symptoms of endometrial cancer appear early in the course, which explains why most women have early-stage disease at presentation. In these cases, the disease is commonly confined to the uterus and can usually be cured by surgery only. The presence of extra-uterine disease significantly affects recurrence rates and survival, emphasizing the importance of identification of sites of disease spread and provision of appropriate adjuvant postoperative therapy.[5] Pathological examination is the cornerstone of diagnosis of endometrial cancer, and different histopathological types of carcinoma can be distinguished. The majority of endometrial cancers are low-grade tumors with endometrioid histology, which have a good prognosis. Endometrioid endometrial carcinoma (EEC) comprises about 80% of all endometrial carcinomas, and typically presents at an early stage, in relatively younger women with obesity, hyperlipidemia, and signs of hyperestrogenism (either endogenous or exogenous). Recurrent disease is usually local, with the pelvis being the most common site, and it is frequently curable with tumor-directed radiotherapy.[4, 6]

SEROUS ENDOMETRIAL CARCINOMA

Uterine papillary serous carcinoma (UPSC) is a highly aggressive subtype of endometrial cancer that represents approximately 10% of all endometrial cancer diagnoses, but is disproportionately responsible for up to 50% of all treatment failures and 40% of all endometrial cancer-related deaths.[7-9] UPSC is histologically similar to serous ovarian carcinoma (SOC) and was first established as a distinct subtype of endometrial cancer in 1981.[10, 11] UPSC was found to have a distinct microscopic appearance, epidemiology, and clinical behavior in comparison with the more common low-risk EEC (Table 1).[12] UPSC most often presents with metastatic disease at the time of diagnosis, is at high risk of recurrence, often arises in lean, older women, and demonstrates no hormonal risk factors.[10, 13, 14] While clinicopathologic risk factors for recurrence and survival have been delineated quite clearly for EEC, prognostic factors such as lymphovascular space invasion (LVSI), myometrial invasion, and tumor size did not impact on recurrence risk or survival in UPSC patients.[8, 9, 15, 16]

Table 1: Contrasting features of endometrioid versus serous endometrial carcinoma.

Feature	EEC	UPSC
Demographics	Younger age (mean age 60 years old) Obesity	Older age (mean age 70 years old) Normal BMI
Risk factors	Hyperestrogenism Obesity Hyperlipidemia	
Pattern of recurrence	Local	Distant
Precursor lesion	Atypical hyperplasia	EIC
Histologic grade	Low, intermediate, or high	High
Molecular changes	*PTEN* gene mutation *KRAS* gene mutation Defective DNA mismatch repair (MSI)	*TP53* gene mutation HER-2/*neu* gene amplification
Stage at presentation	I (73%) II (11%) III (13%) IV (3%)	I (54%) II (8%) III (22%) IV (16%)
Survival by stage	I (85-90%) II (70%) III (40-50%) IV (15-20%)	I (50-80%) II (50%) III (20%) IV (5-10%)

EEC: endometrioid endometrial carcinoma; UPSC: uterine papillary serous carcinoma; MSI: microsatellite instability; BMI: body mass index; EIC: Endometrial intraepithelial carcinoma. Table adapted from Boruta et al.[17]

CLINICAL MANAGEMENT OF UPSC

Uterine papillary serous carcinoma not only resembles SOC morphologically, but also in its clinical behavior, characterized by high recurrence and mortality rates even in early stages of disease. Due to its rarity, most clinicians are unfamiliar with the clinical aspects and management of UPSC. At present, no prospective randomized trials have been performed to establish a standard treatment approach. The existing data are derived exclusively from relatively small retrospective single and multi-institutional studies with limited statistical power and considerable heterogeneity with regard to treatment approach. This inevitably causes uncertainty regarding preferred treatment, and to date, treatment of UPSC is therefore usually a hybrid of the standard approaches for both ovarian and endometrial cancers, incorporating surgery, chemotherapy, and radiotherapy.[17-19]

Surgery & Staging

Endometrial cancer is a surgically staged disease, because clinical estimates and preoperative imaging of the extent of disease are incorrect in over 20% of cases.[20] Therefore, the first introduced clinical staging system for endometrial cancer by the International Federation of Obstetricians and Gynecologists (FIGO)[21] was changed to a surgical staging system in 1988.[20, 22] The similar prognosis of stage IA and IB patients, the uncertainty of defining endocervical glandular involvement, the

recognition of the favorable prognosis in patients with only isolated positive peritoneal cytology, and the poor prognosis in patients with para-aortic lymph node metastasis, led FIGO to revise the 1988 staging system in the year 2009 (Table 2).[23, 24]

Table 2: FIGO staging (2009) for endometrial carcinoma.

Stage I	**Tumor confined to the uterus**
IA	No or less than half myometrial invasion
IB	Invasion equal to or more than half of the myometrium
Stage II	**Tumor invades cervical stroma, but does not extend beyond the uterus***
Stage III	**Local and/or regional spread of the tumor**
IIIA	Tumor invades the serosa of the corpus uteri and/or adnexa$
IIIB	Vaginal and/or parametrial involvement$
IIIC	Metastases to pelvic and/or para-aortic lymph nodes$
Stage IV	**Tumor invades bladder and/or bowel mucosa, and/or distant metastases**
IVA	Tumor invades bladder and/or bowel mucosa
IVB	Distant metastases, including intra-abdominal metastases and/or inguinal lymph nodes

**Endocervical glandular involvement only should be considered as Stage I and no longer as Stage II; $Positive peritoneal cytology has to be reported separately without changing the stage. Adapted from Creasman et al. and Zaino et al.[23, 24]*

The most important therapy for endometrial cancer patients is surgery. The standard surgical procedure includes total hysterectomy including the uterine cervix, and bilateral salpingo-oophorectomy. To date, there is no worldwide consensus whether pelvic and/or para-aortic lymphadenectomy should be performed as part of the staging procedure.[25-27] According to the Dutch guideline, pelvic and/or para-aortic lymphadenectomy should not be performed in patients with EEC and no suspicion of extra-uterine disease. Primary surgery alone is curative in most endometrial cancer patients in whom disease appears to be confined to the uterus on final histopathological examination, as is common in EEC patients. Only 8-13% of the EEC patients have cervical involvement, and about 3-7% will have extra-uterine disease at the time of diagnosis.[28, 29] However, this surgical staging approach routinely used in EEC patients may not be adequate in patients with UPSC. Typically, 55-87% of UPSC patients have metastatic disease at the time of diagnosis[16, 30-32], and approximately 40-70% of clinical Stage I UPSC patients are therefore upstaged at the time of surgical staging.[31-33] Since the clinical behavior and trans-peritoneal spread of UPSC resembles that of SOC, the same surgical management was suggested. This comprehensive surgical staging includes total hysterectomy, bilateral salpingo-oophorectomy, bilateral pelvic and para-aortic lymph node sampling, omentectomy or omental biopsy, and biopsy of any suspect lesions. Various studies have recently emerged showing first data on a favorable impact on survival in comprehensively staged UPSC patients compared to patients treated by a more conservative surgical approach.[30, 31, 34-37] Selectively performing a comprehensive staging procedure in UPSC

patients, based upon uterine factors such as myometrial invasion or LVSI, seemed not reliable in its ability to assess the overall disease status.[33, 38, 39]

Furthermore, questions were raised whether to perform complete debulking surgery with maximum cytoreduction in all stages of UPSC. The beneficial role of complete cytoreduction has already been demonstrated in ovarian cancer patients.[40, 41] Since its close clinical resemblance with SOC, some investigators have addressed the importance of cytoreductive surgery in UPSC patients. Indeed some retrospective studies found a favorable effect of complete cytoreductive surgery on survival, mainly in UPSC patients with advanced stage of disease (FIGO stage III – IV).[34, 42, 43] However, because proper prospective trials are currently lacking, it is still unknown how best to treat patients with UPSC.

Adjuvant treatment

Given the excellent prognosis of early stage low-risk EEC, adjuvant therapy is considered unnecessary in most cases. In case of advanced stage EEC, the mainstay of adjuvant post-operative treatment is radiotherapy. Alternatively, UPSC patients often present with extra-uterine disease and have a poor prognosis with a high recurrence rate, primarily extra-pelvic in nature. As a result, this limits the ability of radiotherapy, as a single modality, to be delivered with curative intent in UPSC patients. Most studies on radiotherapy as adjuvant treatment modality have focused on whole abdominopelvic irradiation (WAPI), whole pelvic radiotherapy (WPRT), and whole abdominal radiotherapy (WART). However, diverging results on the effect of radiotherapy on disease-free and overall survival have been reported.[18, 39, 44, 45] Furthermore, the benefit of vaginal brachytherapy in the adjuvant setting for UPSC patients remains controversial.[46-48] The high frequency of extra-uterine disease at the time of presentation, along with treatment failures within the radiation fields, and the histological and biological similarities between UPSC and SOC, have led to an increasing interest for using chemotherapy as adjuvant treatment modality in UPSC patients. The combination of platinum-based chemotherapy with the addition of taxanes has produced promising results, with significant improvement of recurrence rates and survival outcome for all stages of UPSC.[8, 17, 44, 49-53] However, the benefit of chemotherapy in an adjuvant setting, relative to solely radiation therapy or combined treatment, remains untested in randomized clinical trials in different stages of UPSC disease. Therefore, the optimal adjuvant treatment approach for patients remains elusive.

PREOPERATIVE ASSESSMENT IN UPSC PATIENTS

Endometrial biopsy

Women with endometrial cancer often present with abnormal (e.g. postmenopausal, perimenopausal, or irregular) vaginal bleeding. Histopathological examination has been the cornerstone for the diagnosis of endometrial carcinoma. Endometrial biopsy, performed with an endometrial pipelle in the outpatient clinic, is minimally invasive. It is usually the first step for any woman presenting with abnormal bleeding and currently the standard of care procedure to obtain

a preoperative histopathological diagnosis. Dilatation and curettage (D&C) remains the diagnostic procedure of choice when endometrial biopsy is not feasible or when biopsy specimens are not conclusive for the pathologist.[27, 54] UPSC is known mostly to arise from an atrophic endometrium or from endometrial polyps.[15, 55, 56] False-negative results for both endometrial biopsy and curettage have been found to occur when tumors are localized to a polyp or to a small surface area of the endometrium.[57, 58] Furthermore, preoperative diagnosis of the histological type on biopsies or curettage specimen can be challenging, with up to 20% of the diagnoses of histological type being altered after surgery.[59, 60] In part, this inaccuracy may occur because UPSC is often found admixed with other histological subtypes within an uterine specimen.[59]

Cervical cytology

Investigators have sought for other minimally invasive preoperative methods to detect uterine cancer and to determine high-risk histology or metastatic disease. Already in the 1980's cytopathologists recognized that endometrial cells in cervical cytology were suspicious for endometrial pathology in menopausal women.[61] However, in asymptomatic women, cervical cytology appeared to be a poor screening tool for endometrial carcinoma.[62] Although the incidence of cervical cytology with atypical or malignant endometrial cells is low among patients with endometrial cancer, abnormal cervical cytology was predictive for endometrial pathology and associated with poor prognostic factors.[61-69] It was noticed that UPSC patients may have atypical or malignant endometrial cells within their preoperatively taken cervical cytology more often. Incidence rates have varied though[70-73], and evidence for an association with poor prognostic factors or survival specifically in UPSC patients is lacking.

Serum CA-125

A particularly troublesome feature of UPSC has been the inability to predict extra-uterine spread of disease preoperatively based on the primary diagnostic endometrial biopsy or D&C specimen. Furthermore, uterine prognostic factors such as LVSI, myometrial invasion, and tumor size were invariably not able to predict extra-uterine disease in UPSC patients.[9, 15-17] Since its discovery in 1983[74], the clinical application of the tumor-associated antigen CA-125 has been widely explored. Various studies and prospective trials have demonstrated and validated the clinical utility of serum CA-125 in aspects pertaining to ovarian cancer.[75-78] The histologic similarities between UPSC and SOC and both their propensity for intra-abdominal spread have led investigators to explore the potential role of serum CA-125 to evaluate the disease status in women with UPSC. At present, only a limited number of studies have addressed this issue and there have been conflicting results as to CA-125 and its utility in predicting recurrence.[79, 80] In addition, few studies have explored the role of preoperative serum CA-125 in predicting extra-uterine disease and clinical outcome specifically in women with UPSC. Some found that preoperatively elevated CA-125 was associated with extra-uterine disease at the time of surgery and that it was predictive for reduced survival[81, 82], while others reported that the preoperative level of CA-125 was not predictive for complete or suboptimal

cytoreduction and survival.[83] Further studies are needed to address whether elevated serum CA-125 may be a true surrogate marker for extra-uterine disease and poor clinical outcome in UPSC patients.

HISTOPATHOLOGY OF UPSC

As mentioned earlier, UPSC has a similar morphology as SOC, its homologue in the ovary, and both have a tendency to produce ascites and spread along mucosal and peritoneal surfaces. Since UPSC should be treated more aggressively, it must be distinguished histopathologically from other subtypes of endometrial carcinoma. Histologically, UPSC demonstrates a complex papillary growth pattern, although glandular and solid patterns may also occur, with numerous mitotic figures, fibrovascular micro-papillae and large, branching glands lined by papillary tufts composed predominantly of cuboidal or hobnail cells. Marked nuclear atypia is always present (Figure 1A and 1D).[10, 11, 84] Psammoma bodies are commonly present within the tumor but are not a prerequisite for the diagnosis of UPSC.[10, 55] Recent studies have emphasized that UPSC varies widely in appearance, including tumors composed largely or exclusively of gaping glands with intraluminal papillary proliferations, tumors associated with benign polyps, and mixed tumors in which serous carcinoma coexist with endometrioid, clear cell, or undifferentiated patterns.[55, 84] There is controversy regarding the effect of this mixed versus pure UPSC histology on recurrence risk and survival outcome. It was proposed that mixed tumors, even in which only 10% of the carcinoma appeared serous, usually behave as aggressively as pure UPSC.[10, 19, 36, 55] In contrast, others described a more favorable survival outcome for patients with mixed UPSC compared to pure UPSC.[18] Further studies are needed to elucidate the potential effect of mixed versus pure UPSC histology on recurrence risk and survival outcome.

Precursor of UPSC

The widely accepted pathogenic pathway for low-risk EEC starts with long-lasting unopposed oestrogen exposure leading to endometrial hyperplasia, which increases the chance of development of endometrial hyperplasia with atypia, and eventually of endometrial cancer with endometrioid histology. In contrast, at present less is known about the early events in UPSC carcinogenesis. This is partly the result of the rarity of UPSC compared to its EEC counterpart, and of the probable rapid emergence of UPSC from an apparently normal state with a small window for clinical detection of early disease. Histopathologic studies have suggested that the majority of UPSC develop from a distinctive lesion termed endometrial intraepithelial carcinoma (EIC), which appears to represent malignant transformation of atrophic surface endometrium without myometrial invasion.[55, 85, 86] This was supported by the observations that EIC was often multicentric, topographically related and immediately adjacent to the invasive UPSC (Figure 1B and 1C).[85-87] In addition, the transition from non-neoplastic endometrium to EIC was invariably abrupt, and the presence of EIC in association with UPSC confined to endometrial polyps was found to be suggestive for EIC as an early event in

the pathogenesis of UPSC. EIC was identified in most uteri containing UPSC, but was rarely found in specimens containing other histological subtypes of endometrial cancer.[85-87] The uninvolved endometrium in uteri containing UPSC was usually atrophic and rarely showed endometrial hyperplasia.[15, 55, 85, 86] The incidence of EIC specifically in mixed compared to pure UPSC cases is currently unknown.

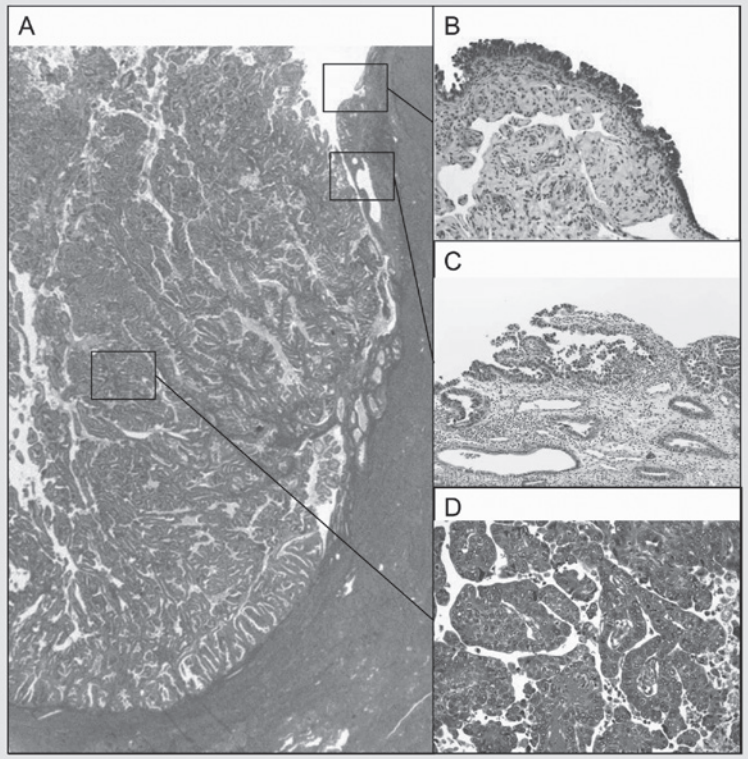

Figure 1: Uterine papillary serous carcinoma (UPSC). **A)** Overview of full-blown UPSC with invasive properties and complex papillary growth (level of magnification: 50x). **B & C)** The distinctive precursor lesion endometrial intraepithelial carcinoma (EIC) represents malignant transformation of atrophic surface endometrium without myometrial invasion, and is immediately adjacent to the invasive UPSC (level of magnification: 200x). **D)** In detail view of UPSC, with its complex papillary growth, numerous mitotic figures, and marked nuclear atypia (level of magnification: 400x).

ORIGIN OF SEROUS OVARIAN CARCINOMA

Ovarian cancer is the most lethal of all gynecological malignancies.[1] Serous ovarian carcinoma (SOC), histologically similar to its endometrial counterpart UPSC, is the most common of the ovarian epithelial malignancies, accounting for approximately 70-80% of cases.[88] The poor prognosis of this disease is attributable to its propensity for serosal organ involvement and peritoneal spread, resulting in up to 60-80% of patients presenting with advanced stage of disease (FIGO stage II-IV).[88, 89] Attempts at screening and early detection of ovarian cancer, using a combination of transvaginal ultrasound, measurement of serum CA-125, and other biomarkers, have largely been unsuccessful.[90-92] Furthermore, early detection is also hindered by uncertainty as to the histologic origin and early natural history of this malignancy. The cell of origin of ovarian cancer has long been debated. Until recently, the paradigm was that epithelial ovarian cancer arises from the ovarian surface epithelium (OSE). OSE is composed of flat, nondescript cells more closely resembling the mesothelium lining the peritoneal cavity, with which it is continuous, rather than the various histologic types of ovarian carcinoma (serous, endometrioid, and clear cell) that have a Müllerian phenotype. Accordingly, the traditional view of ovarian carcinogenesis assumes that ovarian carcinoma originate from the OSE (mesothelium), undergoing a process called metaplasia that accounts for a profound morphologic transformation. Invagination of the metaplastic OSE into the underlying stroma results in epithelial inclusion cysts that eventually undergo malignant transformation.[93-96] However, molecular and clinicopathologic studies have failed to support this hypothesis, and epithelial inclusion cysts were invariably present in both high risk cases and controls.[97, 98] In addition, a precursor lesion was never identified in the ovary itself.

SOC originating in the fallopian tube

In the most widely accepted theory for serous ovarian carcinogenesis to date, the fallopian tube has recently emerged as an important source for female pelvic serous carcinoma. The Müllerian (paramesonephric) tubes are known to develop into the upper part of the vagina, uterus and fallopian tubes during early embryogenesis. The lack of an identified ovarian precursor lesion and the fact that a bilateral salpingo-oophorectomy needs to be performed to protect female *BRCA* mutation carriers for ovarian cancer[99, 100] have led researchers to put forward the fallopian tube as a possible source of SOC. The original lining of the fallopian tube consists of Müllerian type epithelium and malignant transformation of these cells would logically result in a serous carcinoma cell type. Recently, an early form of serous carcinoma in the fallopian tube of female *BRCA* mutation carriers was identified, and this precursor lesion was termed serous tubal intraepithelial carcinoma (STIC).[101-103] It was hypothesized that cells from these lesions may be dislodged and implant in the ovary. However, the incidence of STIC has varied widely, and a spectrum of putative and possibly non-obligate precursor lesions have been described in the fallopian tube.[101, 104-107] Furthermore, the diagnosis and reporting of these lesions may sometimes be problematic with poor diagnostic reproducibility[104, 108], and most studies were lacking a proper control group to differentiate between true premalignant lesions and histological variants of normal tubal epithelium.

SOC originating in the uterus

The histologic and morphologic properties of ovarian carcinoma show striking similarities to those of extra-ovarian tissues that are of Müllerian origin. Interestingly, both tubal ligation and hysterectomy have repeatedly shown a protective effect on the incidence of ovarian cancer.[109-111] UPSC has a very close resemblance to SOC, serous fallopian tube cancer, and other types of pelvic serous cancers, both morphologically and in clinical behavior. A subset of tubo-ovarian serous carcinoma coexist with UPSC of the endometrium. In most cases, multifocal serous carcinomas were presumed to originate from a single rather than multiple sites.[112-114] In addition, although EIC has been described as precursor lesion of UPSC, cases of metastatic disease have been reported in patients with only EIC in their uterus, suggesting that EIC may be, in and of itself, a malignant lesion and not just a precursor.[38, 115, 116] At present, the uterine specimens of SOC patients have never been extensively studied for the presence of a precursor lesion to elucidate the possibility of SOC primarily originating in the uterus.

A paradigm shift resulting in most ovarian and pelvic serous carcinoma being considered to be of fallopian tube or endometrial, rather than ovarian origination could have significant clinical implications in the near future.

OUTLINE OF THIS THESIS

In this thesis we investigate a large cohort of UPSC patients, and focus on preoperative markers and clinicopathological variables whether these can be used as predictors or prognosticators in the clinical management of UPSC. Furthermore, improving care for women with UPSC also requires further understanding of the pathogenesis of this endometrial cancer. We focus on different histopathological aspects of UPSC, including the incidence of the precursor lesion EIC in both pure and mixed UPSC cases.

In **Chapter 2** we review the current literature on the clinical management of UPSC patients, in order to gain more insight whether or not a comprehensive staging procedure should be performed, with or without maximum cytoreduction, as is currently standard practice in ovarian cancer patients. Furthermore, we give an overview of various adjuvant treatment modalities and evaluate the effects of radiotherapy and chemotherapy in UPSC patients.

At present, it remains difficult to predict metastatic disease and survival outcome in UPSC patients already in a preoperative setting. Serum CA-125 has already proven its utility as a prognostic marker, for monitoring the clinical response to treatment, and for the detection of recurrent disease in patients with ovarian cancer. In **Chapter 3**, we investigate whether serum CA-125 can be used as a preoperative tumor marker for the prediction of extra-uterine disease and as prognosticator for survival specifically in patients with UPSC.

Furthermore, in **Chapter 4** we assess whether cervical cytology can be used as predictor for metastatic disease, as prognosticator for survival, and as additional tool in the preoperative work-up of endometrial cancer patients to suspect a high-risk endometrial carcinoma like UPSC.

After final histopathological examination of the surgical specimen, UPSC often shows morphologic heterogeneity containing an additional histological subtype. In **Chapter 5**, we set out to investigate whether pure versus mixed UPSC histology has an impact on recurrence risk and survival. Furthermore, the role of EIC as precursor lesion in the etiology of both pure and mixed UPSC is assessed.

Despite extensive research, never a precursor lesion of SOC has been identified in the ovaries itself. Because STIC in the fallopian tube was recently described as a potential precursor lesion for SOC, in **Chapter 6** we evaluate the fallopian tubes of BRCA mutation carriers at risk for SOC and controls for the presence of this precursor lesion.

Since UPSC and SOC are histopathologically identical, show a similar clinical behavior, and the fact that hysterectomy and tubal ligation had a vast impact on the incidence of SOC, we study the hypothesis of EIC as possible precursor lesion for SOC in **Chapter 7.** The endometrium of patients with SOC is reviewed extensively for the presence of possible premalignant lesions. SOC patients with coinciding EIC in their uterine specimens are selected, and immunohistochemistry as well as *TP53* mutation and DNA ploidy analyses are used to provide first evidence for EIC as lesion of origin for SOC.

Finally, in **Chapter 8** the results of this thesis are discussed in general, and future studies and hypotheses based on the abovementioned studies are proposed.

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Chapter 2

Clinical management of uterine papillary serous carcinoma

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ABSTRACT

Uterine papillary serous carcinoma (UPSC) is an aggressive variant of endometrial cancer. Owing to its rarity, most clinicians are unfamiliar with the clinical aspects and management of UPSC. Furthermore, little prospective evidence exists regarding how best to treat this subset of patients. In anticipation of prospective clinical trials, this review summarizes the latest results of various clinical management options in the different substages of UPSC, with a special focus on the effects of cytoreductive surgery, comprehensive surgical staging and different adjuvant treatment options in relation to recurrence rate and survival outcome.

INTRODUCTION

Endometrial carcinoma is the most common malignancy found in the female genital tract, comprising 40,100 new cases in the USA in 2008, and 1800 new cancers and 400 deaths annually in The Netherlands.[1,101] The majority of endometrial carcinomas (~70–80%) have an endometrioid histology. These tumors typically present at an early stage, have an excellent prognosis and are designated as type I tumors.[2-4] Type II endometrial carcinomas mainly involve serous and clear-cell carcinomas, are poorly differentiated, and have a tendency for deep invasion into the myometrium, a high frequency of extra-uterine metastatic spread and a poor prognosis.[2,5-7]

Uterine papillary serous carcinoma (UPSC) is the prototypical type II carcinoma. It represents only 3–9% of all endometrial cancers, but accounts for up to 39% of all endometrial cancer deaths and is therefore recognized as an unusually aggressive tumor.[1,7-10] Owing to its rarity, most clinicians are unfamiliar with the clinical aspects and management of UPSC. Furthermore, little prospective evidence exists regarding how best to treat this subset of patients. At present, treatment is mainly based on outcomes of retrospective analyses that mostly lack size and power. Over the years, treatment modalities have varied and staging procedures have undergone drastic changes, from clinical to surgical to comprehensive surgical staging. Therefore, extrapolation of the best treatment strategy has been difficult. There is still no consensus on staging procedure and adjuvant therapy, and there is an urgent need for evidence-based recommendations on the clinical management of UPSC. Few randomized clinical trials exist, and none address substages of UPSC specifically in relation to different staging and treatment modalities. We performed a review on the clinical aspects of UPSC with a focus on the effects of staging and adjuvant therapy on recurrence rate and survival outcome in the different (sub)stages of UPSC.

IMPORTANT CLINICAL ASPECTS OF UPSC

The highly malignant potential of UPSC is indicated by a 5-year survival rate of only 18–45%, whereas the overall 5-year survival rate for all stages of type I endometrioid carcinomas is 70–80%.[7,8,10-15] Traditional survival rates for UPSC vary from 35 to 85% in patients with stage I–II disease, and from 0 to 25% in patients with stage III–IV disease.[12,14,16-18]

Several studies have reported on the metastatic properties of UPSC, with a significantly higher incidence of pelvic (41.9%) and para-aortic (43.3%) lymph node metastases and omental involvement (15–35%) compared with endometrioid carcinoma of the uterus.[19,20] Typically, 55–87% of the patients have extra-uterine spreading at the time of diagnosis and approximately 40–70% of clinical stage I UPSCs are therefore upstaged at the time of surgical staging.[14,19,21-23] In addition, 70–83% of all recurrences in UPSC patients occur within 2 years and are associated with abdominopelvic failure and 34% with distant failure.[24,25]

Pathologic review of hysterectomy specimens in UPSC patients often reveals not only pure UPSC (60–75%), but also UPSC mixed with other histologies (25–33%), most often comprising

an endometrioid or clear cell component.[8,26-29] Various studies recently showed that mixed UPSC resembled pure UPSC in prognosis and behavior, with no differences between the two in depth of myometrial invasion, extra-uterine disease or recurrence rate.[16,26,29-34] Importantly, even when as little as 10% of the tumor was composed of papillary serous histology as determined by the pathologist, the patient had the same prognosis and risk for metastases as patients with pure UPSC.[6,29,31] These findings suggest that UPSC is biologically dominant in a heterogeneous tumor and that a serous component, comprising even as little as 10% in a tumor, may dictate tumor progression, metastatic potential and survival outcome.

ROLE OF COMPREHENSIVE SURGICAL STAGING IN UPSC PATIENTS

In 1988, the International Federation of Gynecology and Obstetrics (FIGO) changed the clinical staging system for endometrial carcinoma to a surgical system. However, this standard surgical management used for endometrioid-type carcinoma of the uterus does not appear to be adequate in patients with UPSC, indicated by understaging of stage III–IV UPSC patients.[16,19,35] Since UPSC shows a similar behavior and spread pattern as serous papillary carcinoma of the ovary, comprehensive surgical staging in UPSC patients was suggested to more reliably predict extra-uterine spread.[16,34,36,37] This comprehensive staging includes total abdominal hysterectomy, bilateral salpingo-oophorectomy, pelvic washings, bilateral pelvic and para-aortic lymph node sampling and omentectomy or omental biopsy.

Recently, the possible beneficial role of comprehensive surgical staging in UPSC patients has been shown by a number of studies. Turner *et al.* described stage I UPSC patients with complete comprehensive surgical staging with a significantly better long-term 5-year overall survival (OS) of 95% compared with 45% in stage I UPSC patients without surgical staging.[38] Geisler *et al.* recommended omental evaluation, since 23.8% of all UPSC patients with negative lymph nodes had either omental or peritoneal biopsies that were microscopically positive for metastatic disease.[19] By contrast, Gehrig *et al.* demonstrated that omental sampling may not be necessary in the routine surgical staging of type II carcinoma as the sensitivity of a visually negative omentum was 0.89 ($p < 0.0001$) in their retrospective study of 65 patients.[39] Other investigators indicated that 21–42% of their UPSC patients had evidence of metastatic disease in their sampled omentum or lymph nodes; in some cases this was the only evidence of metastasis, which would otherwise be missed when no comprehensive staging was performed.[19,21,31,35,37] Although comprehensive staging seems to be very important and has major implications in the treatment of UPSC patients, some people have questioned this practice[7,39,40] Goldberg *et al.* were not able to show that comprehensive surgical staging improved the outcome compared with more conservative surgical approaches.[28]

Many studies have demonstrated the unusual propensity of UPSC for extra-uterine spread. Metastases are often found retro- and intraperitoneally, upper abdominally, and there is a high incidence of lymph node and omental involvement.[16,21,26,35,37] Therefore, we feel the necessity of thorough comprehensive surgical staging in UPSC patients cannot be overemphasized. Considering

the high rate of surgical upstaging, the atypical metastatic spread pattern and the limited staging criteria of earlier studies, it seems likely that the poor survival rates commonly associated with stage I–II UPSC disease can be at least partially attributed to initially undetected metastatic disease (i.e., understaging). Based on these observations, comprehensive surgical staging of all UPSC patients can be recommended, regardless of the clinical stage at presentation.

CYTOREDUCTIVE SURGERY IN UPSC TO IMPROVE OVERALL SURVIVAL

The role of optimal cytoreduction, defined as residual disease 1 cm or less in diameter, has already been well demonstrated in ovarian cancer.[41-43] Authors similarly advocated the importance of cytoreduction of all visible metastatic tumor in UPSC patients. Recently, some studies have investigated the effect of aggressive cytoreductive surgery for the first time specifically in UPSC patients.[10,12,19,44,45]

In 2001, Bristow *et al.* investigated the effect of optimal cytoreductive surgery in stage IV UPSC patients. These patients had a median survival of 26.2 months compared with 9.6 months for patients left with suboptimal residual disease ($p < 0.001$).[8] Furthermore, 57.1% of all optimally cytoreduced patients were still alive compared with only 6.7% of patients with suboptimal cytoreduction after 24 months of follow-up. Memarzadeh *et al.* showed that stage IIIC–IV UPSC patients with microscopic residual disease after optimal cytoreductive surgery had a median survival of 40 months, similar to the 43 months for stage IIIA patients who presented only with microscopic disease.[35] This was in sharp contrast to patients with stage IIIC–IV disease with any degree of residual disease, since their survival was only 10 months ($p < 0.001$). Thomas *et al.* described that stage IIIC–IV UPSC patients with no visible residual disease after optimal cytoreduction had a significantly better median survival than those patients with any residual disease left after primary surgery (52 vs 13 months).[46] The 3-year OS of patients with no residual disease was 75%, compared with only 5% for patients with residual disease ($p < 0.001$).

To summarize, there is a growing body of literature suggesting that optimal cytoreductive surgery has beneficial consequences associated with improved survival in stage III–IV UPSC patients. However, surgical treatment as sole therapy in UPSC patients has been shown to be unacceptable, owing to high recurrence rates, mostly in the upper abdomen.[24,26,27,47]

OVERVIEW OF TREATMENT MODALITIES IN UPSC & THEIR OUTCOME

Since UPSC is characterized by high recurrence rates and a poor prognosis, the majority of UPSC patients are offered adjuvant therapy, although optimal treatment for different stages of this type of cancer has remained elusive. Treatment modalities employed during recent decades include pelvic radiation, whole abdominal radiation, vaginal cuff radiation (brachytherapy), different chemotherapy (CT) regimens and combinations of these modalities.

Role of radiation therapy

Radiotherapy has been the most commonly used adjuvant treatment in the management of endometrial cancer. Since UPSC has the propensity to spread to and recur in the pelvic area and upper abdomen, whole abdominopelvic irradiation (WAPI), whole pelvic radiotherapy (WPRT) and whole abdominal radiotherapy (WART) were evaluated as adjuvant treatment. Smith *et al.* described an additional survival benefit of 20–30% in stage I and II UPSC patients with postoperative WAPI after cytoreductive surgery.[48] Bristow *et al.* reported a 5-year OS rate of 64–81% in stage I–II UPSC patients treated with WPRT, although only 31% for stage III patients.[16] Others similarly showed improved long-term survival in stage I–II patients using WPRT, reducing pelvic relapses in these UPSC patients.[12,14]

By contrast, Gallion *et al.* reported recurrences in seven out of nine stage I UPSC patients treated with WPRT, of which six were outside the radiation field.[49] In a study by Frank *et al.,* six out of nine UPSC patients (stage I–III) recurred after treatment with WART after a median follow-up of 7.5 months.[50] Markedly, all recurrences were in-field. In addition, Elit *et al.* found similar recurrence rates in a small cohort of stage I UPSC patients with approximately 50% of the recurrences not in-field.[51] More recently, Slomovitz *et al.* reported on a large UPSC patient cohort with no significant differences in progression-free survival (PFS) and OS between UPSC patients who received adjuvant radiation therapy (RT) after surgery compared with patients who were only observed after cytoreductive surgery.[18] Huh and colleagues evaluated RT in a large group of stage I UPSC patients and the 5-year OS was 66% for the observation group compared with 59% in the RT group.[52] Mehta *et al.* reviewed reports in which WART was used in stage I–II UPSC patients and concluded that the risk for abdominal failure was identical with or without WART treatment, questioning the rationale for WART in these patients.[53] Furthermore, in a prospective study of the Gynecologic Oncology Group (GOG) using WART in stage III–IV UPSC patients, the 3-year OS rate was only 33%.[54] The same investigators also studied the effect of WART in stage I–II UPSC patients and this revealed a 5-year PFS of only 38%, with more than 50% of the recurrences within the radiation field.[55]

There have been diverging reports on the effect of adjuvant RT on survival in UPSC patients. Reasons for dichotomous findings may include variation in radiation technique or in radiation doses, or not all patient populations used in the studies were correctly surgically staged, leading to understaging and worse survival outcome. Either way, adjuvant external radiation was never satisfactory concerning disease control and survival.

Role of brachytherapy

The benefit of brachytherapy in the adjuvant setting for UPSC patients remains controversial. Investigators studying adjuvant RT and systemic CT as treatment modality in UPSC patients have found a high rate of recurrence, typically located at the vaginal apex and intra-abdominally. In 1991, Frank *et al.* reported on a small cohort of UPSC patients treated with WART with or without brachytherapy, in which 50% of the patients who did not receive brachytherapy recurred at the

vaginal apex.[50] In two larger studies, only 6–7% of stage I–II UPSC patients had vaginal recurrences after receiving brachytherapy.[38,56] Kelly and colleagues showed that none of the 38 stage I UPSC patients who received brachytherapy in combination with adjuvant CT recurred at the vaginal cuff, whereas 19% of the stage I patients receiving CT only had local recurrences.[33] Others have confirmed these findings with no recurrences at the vaginal apex when brachytherapy was included in the adjuvant therapy setting, whereas external radiation or systemic CT only resulted in 15–19% vaginal failure.[6,57-60]

Omission of vaginal brachytherapy was associated with a high rate of vaginal apex recurrences, up to approximately 50% in both stage I–II and stage III–IV UPSC patients.[38,48,61] Importantly, although local vaginal cuff recurrence in type I endometrial carcinomas is often salvageable, various studies have reported on unsalvageable tumor progression in UPSC patients at the vaginal apex and most patients died of their recurrent disease.[33,38] In addition, salvage RT for vaginal cuff recurrence is usually more intense and associated with higher toxicity than primary adjuvant brachytherapy. Since brachytherapy is characterized by a relatively low morbidity and results in improved local control and PFS, brachytherapy is recommended to be offered to all UPSC patients in combination with other adjuvant treatment modalities. However, in most recent reports OS was never improved using brachytherapy, probably owing to extensive interabdominal recurrences and distant metastasis, which typically occur in 50–87% of all UPSC patients.[24,33,48,53,57,59]

Role of chemotherapy

Owing to histological and biological similarities between UPSC and serous ovarian cancer, and the positive effect of adjuvant CT in serous ovarian cancer patients, systemic CT was also investigated in UPSC patients. Traditionally, as for all histopathological types of advanced and recurrent endometrial carcinoma, combinations of cisplatin, doxorubicin and cyclophosphamide (CAP) were used. In 1993, Rosenberg *et al.* treated stage I UPSC patients with adjuvant CAP and noticed no recurrences after 32 months.[62] However, others have shown poor response rates of 9–25% in different stages of UPSC disease using CAP, with many patients deceased within 2 years.[63-66] Combined radiation and CT using CAP was also studied, although only a minor improvement in survival in stage I–IV UPSC patients was found compared with patients treated with RT alone.[67] Others also studied the effect of combination therapy using abdominopelvic radiation and CAP in UPSC patients, but results have been disappointing.[68,69] Several authors concluded that UPSC was relatively resistant to this type of CT, in contrast to its serous ovarian counterparts.[63,70,71] Furthermore, significant treatment-associated toxicity in approximately 70% of patients was observed when using CAP with or without external radiation.[63,64]

Zanotti *et al.* and Ramondetta *et al.* were the first to evaluate the potency of paclitaxel alone or in combination with carboplatin in UPSC patients and a response rate of approximately 90% was reported in the adjuvant setting with stage I–IV patients.[66,72] More recently, various reports showed that paclitaxel combined with platinum-based CT was superior in improvement of survival in stage

I–IV UPSC patients compared with platinum-based only or other therapeutic regimen such as CAP.[25,29,33,66,73] In a recent Phase II study, women with stage I–IV UPSC were treated with a 'sandwich' regimen using three cycles of a platinum-based chemotherapeutic agent plus paclitaxel, followed by pelvic RT and another three cycles of CT.[74] This regimen was very well tolerated and PFS and OS were significantly increased for both stage I–II and stage III–IV UPSC patients. By contrast, there have been reports in which no significant effect was observed after treatment of UPSC patients with platinum-based CT, with or without paclitaxel.[18,52] However, these studies included stage I–II UPSC patients without proper surgical staging, possibly resulting in understaging. This could have resulted in underestimation of the survival benefit.

At present, different reports have shown platinum-based CT in combination with paclitaxel to be the most effective adjuvant treatment modality in stage I–IV UPSC patients, and it significantly and favorably impacted upon recurrence rate, PFS and OS.

OPTIMAL MANAGEMENT IN DIFFERENT STAGES OF UPSC

Despite many proposed treatment strategies, including observation only, different radiation and CT modalities and combinations of the former, the clinical management of both early- (stage I–II) and advanced-stage (stage III–IV) UPSC remains controversial. Synthesizing data from previous reports for discerning optimal management in UPSC is challenging for reasons described by Elit *et al.* and Hamilton *et al.*[7,51] Briefly, differences in staging (clinically vs surgically vs comprehensive surgically), treatment outcome (based on combinations of stages due to rarity of disease), limited follow-up time and unclear pathological criteria for the diagnosis of UPSC all make it difficult to distillate treatment outcome and survival numbers specified for each substage.

In this section, we set out to determine the best treatment modality specific for different (sub)stages of UPSC. For papers reporting on outcome after adjuvant treatment, we used a date restriction from January 2000 until present. Interestingly, owing to relatively high total numbers of stage I UPSC patients included in the various studies, we were able to distinguish comprehensively staged patients from patients without proper or with incomplete surgical staging in relation to outcome after treatment. Patients were considered 'fully comprehensively staged' when more than 95% of all patients in the study were staged in this manner. Furthermore, since type and dose of radiation and CT strongly differed in the various included studies, data on WAPI, WPRT, WART and brachytherapy were merged into a single category, RT, whereas data on CT with or without the addition of RT were fused into a separate category (CT ± RT). The staging of UPSC patients in all included studies was based on the FIGO surgical staging system adopted in 1988.

Stage IA

Although evidence for adjuvant platinum-based CT with paclitaxel in advanced-stage UPSC patients seems compelling, the benefit for stage I UPSC patients is still unclear. Various investigators have suggested observation only to be reasonable in stage IA patients after surgery.[33,36,40,59]

However, recurrence rates varied from 0 to 21%, and in most cases recurrences were extrapelvic and not salvageable. External RT did not prove to be beneficial in most studies, resulting in identical recurrence rates to the observation group.[18,33,36,51,73] Adjuvant CT resulted in improved recurrence rates with superior PFS and OS compared with observation only or RT.[33,73,75,76] Our review of the literature reveals that the average recurrence rate in the observation group of comprehensively staged stage IA UPSC patients was approximately 15%, and decreased by more than 50% when these patients were treated with CT with or without RT (Table 1). Observation only and RT were associated with a recurrence rate of 14 and 32%, respectively. The beneficial effect of CT seems to be reduced in noncomprehensively staged patients compared with observation only (Table 2). Interestingly, these data show a difference in recurrence rate in CT with or without RT-treated patients among the differently staged groups. Despite low numbers of patients in both groups that make these findings difficult to interpret, this could possibly correlate with understaging in the noncomprehensively staged patient group.

Stage IB–IC

Stage IB–IC UPSC patients typically show higher rates of recurrence (up to approximately 50%) when only observed or treated with RT compared with stage IA patients.[33,36,59,73,77] This marked difference suggests that the margin of benefit of systemic adjuvant CT could be even more in stage IB–IC UPSC patients. While suggested by some investigators, the high recurrence rates do not seem to justify observation only or RT as the sole treatment modality.[51,78] Systemic CT with or without RT was also evaluated and resulted in decreased recurrence rates and increased PFS and OS.[33,58,59,73-76] After reviewing the literature, we found an average recurrence rate of approximately 35% in the observation only group for both comprehensively staged and noncomprehensively staged stage IB–IC UPSC patients, whereas RT improves the rate of recurrence only to a limited extent (Tables 1 & 2). By contrast, adjuvant CT with or without RT decreases recurrence rates by 40–70% in (non) comprehensively staged patients. Interestingly, the rate of recurrence in noncomprehensively staged stage IB–IC UPSC patients after CT with or without RT is twice as high compared with comprehensively staged patients, possibly correlating with understaging (~21 vs 11%, respectively). Independent of the surgical staging procedure, treatment with CT with or without the addition of RT resulted in a decrease of approximately 50% in recurrence rate in both stage IA and stage IB–IC UPSC patients (~8 and 17%, respectively) (Table 3). In general, the 5-year OS for all stage I UPSC patients was improved by CT with or without RT up to 75–100%, whereas observation only or RT resulted in a survival of 65–80% and 45–65%, respectively.[33,52,59,73]

Table 1: Recurrences in Stage IA and Stage IB-IC UPSC patients by treatment group after comprehensive surgical staging procedure.

		Stage IA						Stage IB-IC					
		OBS		RT		CT +/- RT		OBS		RT		CT +/- RT	
Authors	**Total *N***	***N***	**Recurrence**	***N***	**Recurrence**	***N***	**Recurrence**	***N***	**Recurrence**	***N***	**Recurrence**	***N***	**Recurrence**
Bristow *et al.*[17]	26	4	1	-	-	-	-	6	0	1	1	-	-
Nguyen *et al.*[80]	22	1	0	2	0	-	-	1	0	4	2	-	-
Sood *et al.*[25]	42	-	-	1	0	-	-	-	-	2	1	3	2
Slomovitz *et al.*[19]	129	14	3	5	1	-	-	-	-	-	-	-	-
Chan *et al.*[38]	11	3	1	-	-	2	0	-	-	-	-	-	-
Elit *et al.*[52]	43	19	2	-	-	3	0	8	3	10	3	-	-
Dietrich *et al.*[77]	29	-	-	-	-	7	0	-	-	-	-	22	3
Kelly *et al.*[34]	74	16	2	8	4	9	0	5	4	13	10	23	1
Hamilton *et al.*[37]	57	10	3	-	-	-	-	16	9	-	-	-	-
Thomas *et al.*[41]	42	11	0	3	0	1	0	7	2	14	1	6	0
Kwon *et al.*[79]	22	5	0	-	-	-	-	10	1	-	-	-	-
Goldberg *et al.*[29]	45	-	-	6	3	2	1	-	-	10	2	6	1
Alektiar *et al.*[76]	25	-	-	-	-	7	1	-	-	-	-	13	1
	Total:	83	12	25	8	31	2	53	19	54	20	73	8
	%-recurrence:	**14,5%**		**32,0%**		**6,5%**		**35,8%**		**37,0%**		**10,9%**	

Total N: Total number of patients included in each study, often distributed over different (sub)stages; OBS: Observation only; RT: Radiation therapy; CT +/- RT: Chemotherapy only or combined with radiation therapy.

Table 2: Recurrences in Stage IA and Stage IB-IC UPSC patients by treatment group, without comprehensive surgical staging procedure.

		Stage IA						Stage IB-IC					
		OBS		RT		CT +/- RT		OBS		RT		CT +/- RT	
Authors	**Total *N***	***N***	**Recurrence**	***N***	**Recurrence**	***N***	**Recurrence**	***N***	**Recurrence**	***N***	**Recurrence**	***N***	**Recurrence**
Lim *et al.*[78]	78	4	0	6	3	-	-	10	4	37	10	-	-
Gehrig *et al.*[23]	15	6	2	-	-	-	-	-	-	-	-	-	-
Huh *et al.*[53]	60	-	-	3	1	2	0	-	-	9	1	5	0
Fakiris *et al.*[59]	18	-	-	-	-	3	0	-	-	-	-	10	3
Low *et al.*[58]	26	-	-	-	-	1	0	-	-	-	-	7	1
Havrilesky *et al.*[60]	80	22	1	4	1	6	1	25	6	12	6	11	3
Fields *et al.*[75]	29	-	-	-	-	2	0	-	-	-	-	13	4
Goldberg *et al.*[29]	30	6	2	2	0	1	1	1	1	14	4	6	2
Fader *et al.*[74]	142	19	3	5	1	27	2	14	7	15	4	62	8
Wang *et al.*[46]	40	1	1	-	-	-	-	1	0	-	-	9	5
	Total:	58	9	20	6	42	4	51	18	87	25	123	26
	%-recurrence:	**15,5%**		**30,0%**		**9,5%**		**35,3%**		**28,7%**		**21,1%**	

Total N: Total number of patients included in each study, often distributed over different (sub)stages; OBS: Observation only; RT: Radiation therapy; CT +/- RT: Chemotherapy only or combined with radiation therapy.

Stage II

Approximately 10% of all women with early-stage UPSC have stage II disease, and recurrence rates are generally higher than for stage I patients. Pelvic RT for local control was suggested by some investigators, although WART/WPRT proved to be of only minor benefit with recurrence rates of 25–50%.[16,25,53,77] Substantial data were shown recently by Fader *et al.*, in which stage II UPSC patients treated with CT with or without RT showed improved recurrence rates down to approximately 10%, whereas observation-only and radiation-treated patients had recurrences in approximately 50% of the cases.[25] Despite low patient numbers in the various studies reviewed, stage II patients on average showed a recurrence rate of approximately 40% when observed only after surgery, and RT decreased the recurrence rate only to a minor extent (Table 3). CT with or without RT resulted in an approximately 40% decrease of recurrences, although eventually still more than 20% of all stage II UPSC patients had recurrences after optimal treatment (Table 3). Treatment with CT with or without RT resulted in this group of patients demonstrating an improved 5-year OS of 70–90%, compared with 40–60% in both the observation-only and RT groups.[12,14,25,74,75]

Stage III & IV

Advanced-stage UPSC (stage III and IV) has been characterized by extensive metastatic disease, high recurrence rates after primary treatment and moderate response to adjuvant treatment, all resulting in a poor prognosis and impaired survival. Reviewed literature revealed low numbers of patients in the observation-only and RT groups with a recurrence rate of approximately 70–100% (Table 3). In stage III UPSC patients treated with CT with or without RT, a minor improvement of recurrence rate could be observed (~55%), whereas hardly any effects were found in stage IV patients treated with this modality (Table 3). Unfortunately, CT improved OS rates in advanced-staged UPSC patients only to a limited extent. The 5-year OS rates are still dramatic with 20–50% for stage III and 10–40% for stage IV UPSC patients, although higher than traditional survival numbers of 0–25%.[14,16-18,24,32,57,74,79]

Table 3: Recurrences by substage and treatment group, independent of surgical staging procedure.

	OBS (*N* = 280)		RT (*N* = 274)		CT +/- RT (*N* = 426)		Total recurrences
Stage	***N***	***Recurrence***	***N***	***Recurrence***	***N***	***Recurrence***	**by substage**
IA (*N* = 259)‡	141	21 (14,9%)	45	14 (31,1%)	73	6 (8,2%)	41 (15,8%)
IB-IC (*N* = 441)‡	104	37 (35,6%)	141	45 (31,9%)	196	34 (17,3%)	116 (26,3%)
II (*N* = 134)*	26	10 (38,5%)	62	21 (33,9%)	46	10 (21,7%)	41 (30,6%)
III (*N* = 71)∞	7	5 (71,4%)	17	15 (88,2%)	47	26 (55,3%)	46 (64,8%)
IV (*N* = 78)#	2	2 (100%)	9	7 (77,7%)	67	53 (79,1%)	62 (79,5%)

OBS: Observation only; RT: Radiation therapy; CT +/- RT: Chemotherapy only or combined with radiation therapy.
‡ Calculated for Stage IA and Stage IB-IC after combining the data from Tables 1 and 2.
** Data adapted from: [17,23,25,26,37,38,46,58,59,75,76,78,80]*
∞ Data adapted from: [17,23,25,38,46,58,59,75,78,80]
 # Data adapted from: [9,23,25,33,38,46,58,75,80]

CONCLUSION & DISCUSSION

In this article we summarized the latest findings concerning staging procedure, surgical management and adjuvant treatment options in relation to recurrence rate and outcome in UPSC patients. It now seems advisable to perform a comprehensive surgical staging procedure in both mixed and pure UPSC patients, independent of stage of disease, with a focus on maximum cytoreduction. All patients should undergo total abdominal hysterectomy, bilateral salpingo-oophorectomy, collection of peritoneal fluid for cytologic examination, bilateral pelvic and para-aortic lymph node sampling and omental sampling. In addition, aggressive cytoreductive surgery of all tumor tissue seems warranted to enhance response to adjuvant therapy. Recent data show major improvements in OS and recurrence rates in all stages of UPSC, including stage IA patients, when patients were treated with a combination of CT with or without RT with the addition of brachytherapy to decrease vaginal recurrences.

Although the optimal adjuvant treatment for different stages of UPSC remains to be determined in prospective randomized clinical trials, our literature review raises some questions regarding the use of RT other than brachytherapy in UPSC patients. Whole abdominal and pelvic radiation may not be necessary in either early- or advanced-stage UPSC patients. Most studies are not powered to determine whether the addition of pelvic or abdominal radiation to CT resulted in improved recurrence rate and survival outcome. Furthermore, recurrences in patients treated with postoperative RT are most likely to be distant and result in poor overall prognosis. Our review of the literature revealed that RT in most cases does not result in a decrease of recurrences among UPSC patients, and thus it seems that RT alone is insufficient as an adjuvant treatment modality. Finally, there is some evidence that UPSC is intrinsically less sensitive to irradiation than other histologic types.[80] UPSC is characterized by *TP53* mutations, and overexpression and mutations in and/or loss of functioning p53 protein have been implicated in increased cellular radioresistance.[80,81] In addition, the gene *HER2* is commonly overexpressed by UPSC and *HER2* overexpression in breast cancer has been correlated with decreased radiosensitivity.[82] This could be consistent with the low efficacy for WART, WPRT and WAPI observed in the adjuvant setting for UPSC patients. Therefore, we suggest that external RT should not be used as the single curative treatment modality. This will also spare patients the morbidity associated with treatment using a large radiation field.

Unlike ovarian cancer, UPSC has been demonstrated to be relatively chemoresistant. The effect of CAP CT on the outcome of patients with UPSC has been controversial, and the results of platinum-based CT as sole treatment have been disappointing.[50,61,63,64,67] The addition of paclitaxel to platinum-based CT produced promising results, with significant improvement of recurrence rates and survival outcome for all stages of UPSC.[15,66,71] Our literature review suggests that a combination of platinum-based CT with paclitaxel decreases recurrence rates by 25–70% depending on stage of disease, and improves 5-year OS outcome up to approximately 50% in stage III UPSC patients and up to 70–100% in patients with stage I–II disease. Importantly, paclitaxel is very well tolerated, with acceptable toxicity, and it has been shown to retain some activity in patients heavily pretreated with other chemotherapeutic regimens.[66,71,83]

Since UPSC typically shows recurrences intra-abdominally and more importantly at the vaginal apex, the relevance of brachytherapy has become more and more accepted. Although brachytherapy has never been used as the single adjuvant treatment modality in UPSC patients, it is possible that a large proportion of the recurrences at the vaginal apex could be overcome by brachytherapy. We have to consider though that brachytherapy does not improve OS in most studies, mainly owing to distant metastases. However, the decrease in vaginal recurrences resulted in enhanced local control and in improved PFS.

A particularly troublesome feature of UPSC has been the inability to predict extra-uterine spread based on the primary tumor pathology. Myometrial invasion and tumor grade have been shown to be of no prognostic value.[11,21,26,37] This highlights the importance of comprehensive surgical staging to prevent understaging, since up to approximately 70% of all UPSC patients are upstaged during primary surgery.

Specific recommendations regarding management of the different substages of UPSC have varied widely owing to its low incidence, the variety of surgical interventions and the wide variety of postoperative adjuvant therapies. Some authors have recommended clinical follow-up only after surgery, particularly in stage IA patients.[16,33,84,85] In addition, Kwon *et al.* showed that in stage IA–IB UPSC patients, after complete surgical staging without further adjuvant treatment, only one out of 22 patients recurred.[78] By contrast, others have recommended aggressive adjuvant therapy using both postoperative CT and RT.[38,62,67,68] Tables 1 & 2 suggest that stage IA UPSC patients still have a relatively high recurrence rate (up to 15%) and worse survival when observed only or treated solely with RT. Our review reveals that stage IA–IV UPSC patients could all potentially benefit from comprehensive surgical staging with maximum cytoreduction, followed by platinum-based CT combined with paclitaxel with optional brachytherapy.

A novel approach to the treatment of UPSC patients is the use of neoadjuvant CT (NACT) prior to surgery and adjuvant CT. Data have been accumulated using NACT in advanced stages of epithelial ovarian cancer and recently NACT prior to primary staging surgery of UPSC patients was investigated in a limited number of studies with promising results: the use of NACT resulted in a high rate (80%) of optimal interval debulking surgery of patients with transperitoneal spread of disease.[86] Furthermore, reduction of residual disease prior to surgery was correlated with better survival outcome and improved quality of life by reduction of complications.[86,87] Further research will be necessary to validate the possibly beneficial role of NACT prior to primary surgery in the treatment of UPSC patients.

There are several limitations of this review, mostly inherent to the retrospective design of the majority of included studies and the rarity of UPSC. Furthermore, single-institution or cooperative group trials typically included all endometrial cancer histologies or analyzed combined substages of UPSC, leaving subset analysis of UPSC inadequate to distinguish between arms of therapy or between different substages of disease. Among the reviewed studies, there was a large variation in staging procedure (clinically vs surgically vs comprehensively surgical) and a wide variety of (combined) adjuvant treatment modalities. Treatment groups were often not matched for various variables, including age,

and there were major differences in median follow-up time. These limitations make the results of the various studies somewhat hard to interpret, and without proper prospective randomized trials, no definite conclusions can be drawn.

EXPERT COMMENTARY

At present, comprehensive surgical staging with maximal cytoreduction seems to be essential for optimal management of UPSC, overcoming upstaging with improvement of recurrence rates and survival outcome. In light of the significant upstaging often required when comprehensive staging was not performed, it is reasonable to postulate that this 'stage migration' explains the previously reported poor survival rates of 35–50% for stage I–II UPSC patients. Maximum cytoreduction will enhance response to adjuvant therapy and provides a favorable survival rate. Even though optimal treatment for UPSC remains elusive, external RT seems not to be of any significant value for survival and is often accompanied by severe toxicities. Brachytherapy has been demonstrated to be of minor importance for OS, although it does improve local control and PFS and thus should be considered. CT has been shown to be beneficial in all (sub)stages of UPSC disease, reducing local and distant recurrences. Combinations of platinum with paclitaxel have shown the most promising survival outcome.

Five-year view

Comprehensive staging with maximal cytoreduction followed by adjuvant CT seems more and more warranted in UPSC patients. However, most data on the clinical management of UPSC patients are based on small, retrospective, single-institution studies that are plagued by the usual biases and confounders. In 2006, an international prospective clinical trial (Post Operative Radiotherapy for Endometrial Carcinoma [PORTEC]-3) was initiated to study the effect of RT alone compared with RT with the addition of adjuvant CT in endometrial carcinoma.[102] However, despite the fact that UPSC patients are included in this prospective clinical trial, we argue that this study will not reveal the optimal adjuvant treatment option specifically for UPSC patients, because 1) It is not mandatory to perform a comprehensive staging procedure after which UPSC patients can be included in this study; 2) There is an ethical dilemma since UPSC patients will be randomized and thus can end up in the RT arm of the study, most likely not the most effective treatment modality at present; and 3) The number of included UPSC patients will be relatively low owing to its rarity, resulting in a lack of power to perform statistical analysis in both treatment arms on survival outcome specifically in these patients. Therefore, we feel there is an urgent need for a prospective randomized clinical trial with international collaboration, specifically for UPSC patients. In this trial, we suggest that all patients should be comprehensive surgically staged, with maximum cytoreductive surgery, followed by randomization in one of the two following arms of adjuvant therapy: standard treatment (observation only or RT, depending on stage of disease) compared with CT (in all stages of UPSC disease, including stage IA). This trial should mainly focus on stage I and II UPSC patients since these patients will possibly benefit most from using CT.

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Chapter 3

Preoperative CA-125 predicts extra-uterine disease and survival in uterine papillary serous carcinoma patients

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ABSTRACT

Objective

We determined the clinical utility of preoperative serum CA-125 as predictor of extra-uterine disease and as prognosticator for survival in patients with uterine papillary serous carcinoma (UPSC).

Methods

Patients diagnosed with UPSC, identified between 1992 to 2009, and with preoperative CA-125 measurement were included. A receiver operator characteristic (ROC) curve was used to quantify marker performance. Overall and progression free survival were analyzed using the Kaplan-Meier method. Regression analyses were used to investigate the association of preoperative CA-125 levels and other clinicopathologic variables with the presence of extra-uterine disease and for the effects on survival.

Results

66 patients met the study criteria. Using ROC, 45 U/mL as cut-off level provided the best sensitivity and specificity (75% and 74%, respectively) for extra-uterine disease, with a positive predictive value of 86%. Survival was significantly longer in patients with preoperative CA-125 ≤ 45 U/mL ($p < 0.001$). Only preoperative CA-125 > 45 U/mL remained significantly associated with extra-uterine disease (OR 6.30, 95% CI 1.93 – 20.62). Furthermore, advanced FIGO stage (HR 4.53, 95% CI 1.50 – 13.62) and preoperative CA-125 > 45 U/mL level (HR 3.12, 95% CI 1.13 – 8.73) were associated with decreased survival.

Conclusion

Preoperative elevated serum CA-125 is an independent predictor for the presence of extra-uterine disease and an independent risk factor for survival in UPSC patients.

INTRODUCTION

Uterine papillary serous carcinoma (UPSC) is a highly malignant subtype of endometrial adenocarcinoma, representing only 10% of all uterine carcinomas. However, it accounts for up to 40% of all endometrial cancer deaths and was therefore recognized as an unusually aggressive tumor.[1,2] Unlike its endometrioid adenocarcinoma counterpart, UPSC patients commonly present with advanced stage of disease. UPSC has a high propensity for lymphovascular space invasion and metastasis, even when diagnosed without myometrial invasion.[3-5] UPSC shows a similar behavior and spread pattern as serous papillary carcinoma of the ovary and morphologically these two entities are identical.[2,6] The highly malignant potential of UPSC is also indicated by a 5-year overall survival of only 18-45%.[5,7] Typically, 55-87% of the women with UPSC will have disease spread outside of the uterus (i.e. extra-uterine disease) at time of diagnosis and approximately 40-70% of clinical stage I UPSC are therefore upstaged at the time of surgery.[8-11]

A particularly troublesome feature of UPSC has been the inability to predict extra-uterine spread of disease based on the primary diagnostic dilatation & curettage specimen. In addition, tumor grade is of no prognostic value and sensitivity of CT and MRI for detecting extra-uterine disease is limited.[8,12,13] Comprehensive surgical staging instead of classical staging has been suggested to more reliably determine extra-uterine spread of disease in UPSC patients.[6,11,14,15] This staging procedure should include hysterectomy, bilateral salpingo-oophorectomy, peritoneal washings, bilateral pelvic lymph node dissection with para-aortic lymph node sampling and omental biopsy or omentectomy. Investigators already showed that 21-42% of the UPSC patients had metastatic disease in their sampled omentum or lymph nodes, in some cases even as the only evidence of metastasis and otherwise missed when no comprehensive staging was performed.[10,15,16] However, this comprehensive staging procedure requires more surgical expertise and is associated with higher complication rates. A preoperative test or tumor marker that can reliably discriminate between early and advanced stages of UPSC would therefore be helpful in the clinical management and planning, and possibly in prognostication.

The tumor-associated antigen CA-125 level has proven its utility in epithelial ovarian cancer (EOC). Many prospective trials have validated the use of CA-125 in EOC patients as a prognostic marker, for monitoring the clinical response to treatment and for the detection of recurrent disease.[17-19] However, serum CA-125 levels were not predictive for the presence of advanced stage of disease in EOC patients.[17-19] The utility of preoperative serum CA-125 measurement in endometrial cancer patients has also been investigated. Preoperative serum CA-125 elevations in endometrial cancer patients were first described in patients with recurrent and advanced carcinoma of the uterine corpus.[20] Several studies have shown elevated preoperative CA-125 levels being associated with different clinicopathologic risk factors, extra-uterine tumor spread and reduced actuarial survival.[21-24] However, most of these studies included different histological tumor types and lacked proper review by an experienced gyneco-pathologist. Furthermore, very few studies addressed the utility of preoperative CA-125 measurement solely in UPSC patients.[25-26]

The purpose of this study was to determine the utility of preoperative serum CA-125 as a predictor of extra-uterine disease and as a prognostic factor for survival in patients diagnosed with UPSC.

MATERIALS & METHODS

Patient selection

A retrospective multi-centre study was started by identifying all UPSC patients between January 1992 and December 2009. A total of 141 patients were identified which had primary surgery at one of the five participating hospitals in the eastern region of the Netherlands (Radboud University Nijmegen Medical Centre, Rijnstate Hospital Arnhem, Gelderse Vallei Hospital Ede, Canisius Wilhelmina Hospital Nijmegen and Maas Hospital Boxmeer). Pathological, medical and operative records of all 141 patients were retrieved from the hospitals involved.

All histopathological slides were reviewed by an expert gyneco-pathologist (JB) verifying the diagnosis pure or mixed UPSC. The tumor was considered pure UPSC when the serous component in the carcinoma comprised >75% of the total carcinoma and denoted as mixed UPSC when the serous component comprised between 10-75% of the total carcinoma. When the serous component did not comprise a significant proportion of the tumor (<10%), the carcinoma could not be designated as mixed UPSC according to previously published criteria [27], and these patients were excluded from this study ($N = 26$). In the participating hospitals, serum CA-125 was similarly measured with immunoassay's on the AxSym analyzer, Abbott Diagnostics. For the preoperative workup of UPSC patients it has never been mandatory to include CA-125 measurement and therefore 49 patients had to be excluded since preoperative serum CA-125 levels were unknown. Thus a total of 66 patients were included in this study.

All patients underwent surgical treatment, except for 5 patients because of serious comorbidity ($N = 4$) or because the patient refused treatment ($N = 1$). Three out of these five patients had stage IV disease based on metastases in liver ($N = 1$), brain ($N = 1$) and lung ($N = 1$), the other two had stage III disease based on cervical and vaginal involvement without further evidence of metastatic disease. These 5 patients remained to be included in our study to determine the preoperative marker performance of serum CA-125, however these patients did not receive optimal surgical and adjuvant treatment and were excluded from our further analyses on survival. Patients were staged according to the 2009 FIGO surgical staging system. Surgical treatment included peritoneal washing, total abdominal hysterectomy and bilateral salpingo-oophorectomy, with/without pelvic and para-aortic lymph node sampling or lymphadenectomy and omentum sampling. Clinicopathological data were collected regarding preoperative serum CA-125, age, parity, BMI, FIGO stage, mixed versus pure serous histology, lymphovascular space invasion, myometrial invasion, tumor diameter and extra-uterine disease. Tumor diameter was analyzed as both a continuous and categorical variable, whereas extra-uterine disease was defined as spread of disease outside the uterine corpus, excluding direct growth into the cervix.

Statistical methods

To quantify the marker performance of CA-125 for the detection of extra-uterine spread of disease and to analyze the sensitivity, specificity and the positive and negative predictive values of preoperative serum CA-125 levels at different cut-off values, a receiver operator characteristic (ROC) curve was constructed. The optimal cut-off value of preoperative CA-125 in relation to extra-uterine disease was determined using the Youden's *J* index.[28] Note that this index is based on the principle of equal costs of misclassification, because the loss or gain in sensitivity and specificity is weighted equally. Furthermore, univariable logistic regression analysis was performed to investigate the association of different clinicopathologic variables with the presence of extra-uterine disease at the time of diagnosis. Variables reaching a level of statistical significance were entered into a multivariable logistic regression model with stepwise selection procedures to identify variables that independently predict the presence of extra-uterine disease. A significance level of 0.05 was used to remain in this model.

Length of overall survival (OS) was calculated from the date of initial surgery to the date of death or of last contact, whereas surviving patients were censored at the date of last contact. Progression free survival (PFS) was defined as the time in months from initial surgery to the date of recurrence. Overall and progression free survival estimates were plotted utilizing the Kaplan-Meier method. To examine the effects of various clinicopathogical variables on PFS and OS, univariable analyses were performed using a Cox proportional hazard method. Variables reaching a level of statistical significance in univariable analysis were valid for entry into a multivariable Cox proportional hazards model with stepwise selection procedures. Using a significance level of 0.05 to remain in the model, this multivariable Cox proportional hazards model was used to identify independent risk factors for survival. All statistical analyses were performed using SPSS version 16.0 for Windows (SPSS, Inc, Chicago, IL). $P < 0.05$ was considered statistically significant.

RESULTS

Demographic and histopathologic characteristics of the 66 UPSC patients included are presented in Table 1. The median age at diagnosis was 70 years, with a range from 51 to 87. Eighteen patients (27.2%) had stage I disease, 5 (7.6%) had stage II disease, 14 (21.2%) had stage III disease and 29 (43.9%) had stage IV disease. Twenty-four out of these 29 stage IV patients already had gross macroscopic disease at the time of surgery. In 30 UPSC patients lymph nodes were sampled: all 30 patients received a pelvic lymphadenectomy, whereas 13 UPSC patients received both a pelvic and para-aortic lymphadenectomy. Among these 30 patients with lymph nodes sampling, 15 (50.0%) had nodal metastases. To note, among patients without proper lymph node sampling, 26 out of 36 patients (72.2%) had obvious disseminated disease based on macroscopic tumor deposits, bone or lung metastases or peritoneal disease. Furthermore, lymphovascular space invasion was present in 34 women (54.8%), tumor invasion >50% of the myometrium was present in 32 patients (52.5%), and the median diameter of the tumor was 4.0 cm (range 0.5 – 15.0). Histology was verified as pure UPSC

in 42 patients (63.6%), while 24 patients (36.4%) had a mixed UPSC. Markedly, 47 patients (71.2%) had extra-uterine disease at the time of primary surgery after staging and pathologic evaluation. Twenty-three UPSC patients (34.8%) did not receive adjuvant treatment after surgery, whereas 23 patients (34.8%) received adjuvant radiotherapy and 20 patients (30.4%) adjuvant chemotherapy. Among patients not receiving adjuvant treatment, five did not receive surgery due to serious comorbidity or patient refusal. Of those patients treated by surgery only, 10 of 18 patients had stage I-II disease and 8 of 18 patients had stage III-IV disease. To note, most of the stage III-IV patients refused further adjuvant treatment, and had disseminated disease with spread to mediastinum, lungs, or peritoneal disease, with a dismal prognosis.

Table 1: Patient characteristics of the total study population (*N* = 66).

Variables	**Median (range) / *N* (%)**	
Age at diagnosis (years)	70 (51 - 87)	
Follow-up (months)	17.5 (1 - 163)	
BMI (kg/m^2)	27.0 (18.8 - 35.8)	
Parity (number)	2 (0 - 7)	
Preoperative CA-125 (U/mL)	55.5 (2.0 - 13.000)	
Menopausal state		
Premenopausal	4	(6.1%)
Postmenopausal	62	(93.9%)
FIGO Stage[‡]		
I	18	(27.2%)
II	5	(7.7%)
III	14	(21.2%)
IV	29	(43.9%)
Peritoneal Cytology		
Negative	25	(43.1%)
Positive	33	(56.9%)
Not sampled	8	
Para-aortic and/or Pelvic Lymph nodes		
Negative	15	(50.0%)
Positive	15	(50.0%)
Not sampled[a]	36	
Extra-uterine spreading		
No	19	(28.8%)
Yes	47	(71.2%)
Cervix involvement / Total sampled	27/63	(42.9%)
Vagina involvement / Total sampled	4/66	(6.1%)
Ovaries and fallopian tube involvement / Total sampled	25/63	(39.7%)

Table 1 (continued)

Variables	**Median (range) / *N* (%)**	
Lymphovascular invasion		
No	28	(45.2%)
Yes	34	(54.8%)
Unknown	4	
Histology		
Pure UPSC	42	(63.6%)
Mixed UPSC	24	(36.4%)
Myometrial invasion		
≤1/2 myometrium	29	(47.5%)
>1/2 myometrium	32	(52.5%)
Unknown	5	
Diameter of tumor (cm)	4.0	(0.5 - 15.0)
Adjuvant treatment		
None	23	(34.8%)
Radiotherapy	23	(34.8%)
Chemotherapy	20	(30.4%)

[a]Lymph node status assigned from inspection/palpation at laparotomy or from imaging; [‡]Stage of disease was adapted according to the 2009 FIGO surgical staging system; FIGO: International Federation of Obstetrics and Gynecologists; UPSC: uterine papillary serous carcinoma.

Preoperative serum CA-125 and extra-uterine disease

The median preoperative serum CA-125 level was 55.5 U/mL with a range of 2.0 - 13.000 U/mL. In patients with extra-uterine disease the median preoperative serum CA-125 level was significantly higher (median 124.0 U/mL; 25th-75th quartiles 28.0 - 433.9 U/mL) compared to patients without extra-uterine disease (median 17.5 U/mL; 25th-75th quartiles 10.1 – 28.5 U/mL) ($p < 0.001$). We set out to determine whether preoperative serum CA-125 levels were able to distinguish extra-uterine disease from local disease in our UPSC population. Preoperative CA-125 measurement as predictor for extra-uterine disease had an area under the curve (AUC) of 0.76 (95% CI, 0.64-0.88) in ROC analysis. The cutoff value was chosen to be 45 U/mL based on the equal costs of misclassification principle (Table 2). This resulted in a sensitivity and specificity for predicting extra-uterine disease of 75% and 74% respectively, with a positive predictive value of 86%. Table 2 shows sensitivity, specificity, PPV and NPV at different CA-125 cut-off levels.

Table 2: The sensitivity, specificity and the positive and negative predictive values at different cut-off values of preoperative CA-125 levels for detection of extra-uterine disease in UPSC patients.

CA-125 Cut-off (U/mL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
35	75	47	78	43
40	75	63	85	51
45	**75**	**74**	**86**	**52**
50	72	74	87	52
55	68	84	94	51

PPV: positive predictive value; NPV: negative predictive value.

Lymphovascular invasion and preoperative serum CA-125 > 45 U/mL were both significantly associated with the presence of extra-uterine disease in univariable analysis (OR 4.04 (95% CI, 1.28 – 12.81); and OR 6.32 (95% CI, 1.96 – 20.33) respectively) (Table 3). Importantly, age at diagnosis, BMI, pure or mixed histology, depth of myometrial invasion and tumor diameter (both as continuous or categorical variable) were not associated with extra-uterine spread of disease. Preoperative serum CA-125 > 45 U/mL remained significantly associated with the presence of extra-uterine disease in multivariable analysis. Adjusted for covariates, UPSC patients with a preoperative CA-125 level > 45 U/mL have a 6.30 (95% CI, 1.93 -20.62) times greater risk for extra-uterine disease (Table 3).

Table 3: Crude and Adjusted Odds ratios (OR) with 95% confidence interval (CI) for the presence of extra-uterine disease by clinicopathological variable of UPSC patients, using uni- and multivariable logistic regression with selection procedure.

		Univariable	Multivariable
Variable	***N***	**OR (95% CI)**	**OR (95% CI)**
Age at diagnosis (years)	66	0.96 (0.91 – 1.02)	NS*
BMI (kg/m^2)	59	1.06 (0.91 – 1.23)	NS*
CA-125			
≤45 U/mL	25	1.00 (reference)	1.00 (reference)
>45 U/mL	41	**6.32 (1.96 – 20.33)**	**6.30 (1.93 – 20.62)**
Histology			
Pure UPSC	42	1.00 (reference)	NS*
Mixed UPSC	24	0.52 (0.18 – 1.55)	
Lymphovascular invasion			
No	28	1.00 (reference)	NS*
Yes	34	**4.04 (1.28 – 12.81)**	
Myometrial invasion			
≤1/2 myometrial	29	1.00 (reference)	NS*
>1/2 myometrial	32	0.99 (0.33 – 2.93)	
Maximum tumor diameter			
≤4cm	31	1.00 (reference)	NS*
>4cm	24	0.70 (0.22 – 2.24)	

**NS: Not selected because it was dropped out during stepwise multivariable analysis.*

Survival analysis

Median follow-up was 17.5 months with a range of 1 to 163 months, while the median recurrence interval was 10 months with a range of 2 to 34 months. During the follow up period, 31 patients (47.0%) developed a recurrence and 17 patients (25.8%) suffered from progression of disease despite treatment. Forty-two patients (63.6%) eventually died of UPSC and 4 (6.1%) died of other causes. Figure 1A demonstrates Progression Free Survival (PFS) and Figure 1B Overall Survival (OS) when patients were grouped using preoperative serum CA-125 level of 45 U/mL as a cut-off. The median PFS for patients with preoperative CA-125 ≤ 45 U/mL was not reached during the duration of this study, whereas the median PFS for patients with CA-125 > 45 U/mL was 4 months ($p < 0.001$). In addition, OS was significantly shorter in the group of UPSC patients with preoperative CA-125 > 45 U/mL (median 11 months) compared to the group with preoperative serum CA-125 ≤ 45 U/mL (median 28 months) ($p < 0.001$).

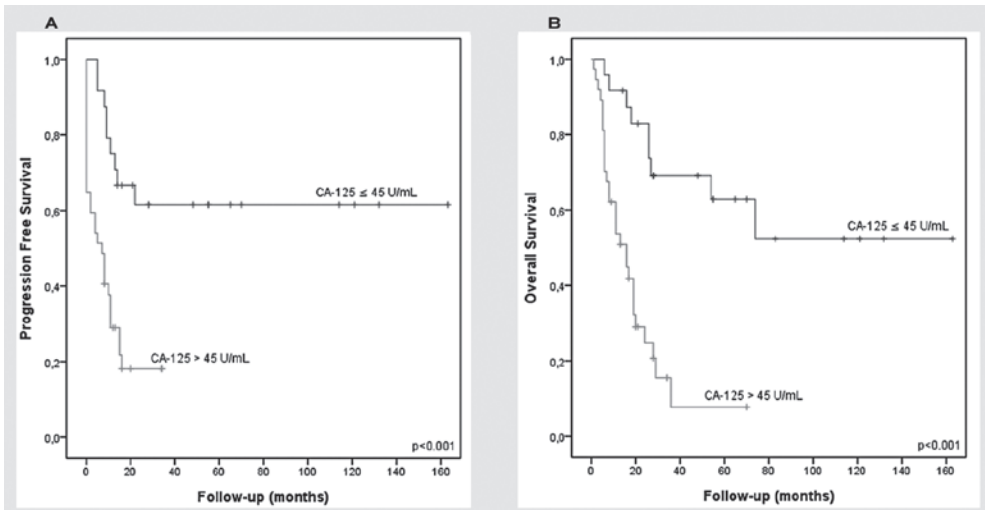

Figure 1: Kaplan-Meier estimates of **A)** Progression Free Survival (PFS) and **B)** Overall Survival (OS) of uterine papillary serous carcinoma patients with preoperative serum CA-125 ≤ 45 U/mL ($N = 24$, upper line) and patients with serum CA-125 > 45 U/mL ($N = 37$, lower line) ($p < 0.001$, for both PFS and OS). Vertical bars indicate patients with censored data.

In a Cox proportional hazard model the clinicopathologic variables were analyzed for their association with PFS and OS. In univariable analyses, FIGO stage and CA-125 > 45 U/mL were significantly associated with PFS, whereas FIGO stage, CA-125 > 45 U/mL and lymphovascular invasion were significantly associated with OS (Table 4). In contrast, no significant associations were found between survival and the diameter of the tumor (as both a continuous or categorical variable), depth of myometrial invasion or pure versus mixed histology of the tumor.

Table 4: Crude hazard ratios (HR) with 95% confidence interval (CI) of Progression Free Survival (PFS) and Overall Survival (OS) by clinicopathological variable of UPSC patients, using univariable Cox regression.

		PFS	OS
Variable	***N***	**HR (95% CI)**	**HR (95% CI)**
Age at diagnosis (years)	61	0.99 (0.95 - 1.02)	0.99 (0.95 - 1.02)
BMI (kg/m^2)	55	1.01 (0.92 - 1.12)	1.02 (0.91 - 1.14)
CA 125			
≤45 U/mL	24	1.00 (reference)	1.00 (reference)
>45 U/mL	37	**3.81 (1.77 – 8.21)**	**5.20 (2.29 - 11.80)**
FIGO Stage			
I	18	1.00 (reference)	1.00 (reference)
II	5	1.91 (0.48 – 7.65)	2.04 (0.51 – 8.20)
III	12	1.79 (0.58 – 5.55)	1.76 (0.56 - 5.50)
IV	26	**6.02 (2.38 – 15.24)**	**8.86 (3.38 – 23.19)**
Histology			
Mixed	23	1.00 (reference)	1.00 (reference)
Pure UPSC	38	1.70 (0.83 – 3.51)	1.71 (0.82 - 3.54)
Lymphovascular invasion			
No	27	1.00 (reference)	1.00 (reference)
Yes	34	1.82 (0.90 – 3.65)	**2.07 (1.03 – 4.16)**
Myometrial invasion			
≤1/2 myometrial	29	1.00 (reference)	1.00 (reference)
>1/2 myometrial	32	1.07 (0.54 - 2.09)	1.09 (0.55 – 2.14)
Maximum tumor diameter			
≤4cm	31	1.00 (reference)	1.00 (reference)
>4cm	24	0.89 (0.45 – 1.77)	1.17 (0.59 – 2.34)

Using multivariable survival analysis, both advanced FIGO stage and preoperative CA-125 > 45 U/mL were significantly and independently associated with both reduced progression free and reduced overall survival (Table 5). To note, inclusion of the 5 previously excluded patients not receiving optimal surgical and adjuvant treatment did not alter our results on survival significantly (data not shown).

Table 5: Adjusted hazard ratios (HR) with 95% confidence interval (CI) of Progression Free Survival (PFS) and Overall Survival (OS) by clinicopathological variable of UPSC patients, using multivariable Cox regression with selection procedure.

		PFS[#]	OS[#]
Variable	***N***	**HR (95% CI)**	**HR (95% CI)**
CA 125			
≤45 U/mL	24	1.00 (reference)	1.00 (reference)
>45 U/mL	37	**2.56 (1.08 – 6.43)**	**3.12 (1.13 - 8.73)**
FIGO			
I	18	1.00 (reference)	1.00 (reference)
II	5	2.10 (0.58 – 11.49)	1.83 (0.45 - 7.42)
III	12	1.68 (0.76 – 7.61)	1.59 (0.51 – 4.95)
IV	26	**3.56 (1.23 – 9.59)**	**4.53 (1.50 – 13.62)**

[#]FIGO stage, Serum CA-125, and LVSI were entered in the multivariable model.

DISCUSSION

A preoperative marker that can reliably discriminate between early and advanced stages of UPSC and can predict survival outcome would be helpful in the clinical management and prognostication of UPSC patients. In this largest study in literature evaluating the possible role of preoperative serum CA-125 level exclusively in UPSC patients, we demonstrated that preoperatively elevated serum CA-125 was the only studied factor significantly associated with extra-uterine disease. In addition, an elevated preoperative serum CA-125 level was, together with stage of disease, a significant independent predictor of decreased survival.

Unlike the typical endometrioid adenocarcinoma of the uterine corpus, UPSC commonly presents at an advanced stage and even when diagnosed without myometrial invasion it may be associated with disseminated disease.[3,4] In the present study, 71% of the UPSC patients had extra-uterine disease at the time of diagnosis, consistent with previously reported data of 55-87% of the women having extra-uterine disease.[8-10] Within the clinical setting, the assessment of serum CA-125 may be a useful test during the initial evaluation of a patient with UPSC. First, we demonstrated a strong association of serum CA-125 elevation with extra-uterine disease. More importantly, elevated CA-125 was the only studied preoperative factor significantly associated with disseminated disease. Although the management of all stages of UPSC should include surgical staging, preoperatively elevated serum CA-125 may prompt to use imaging to detect sites of metastatic disease that may not be clinically apparent. Moreover, the preoperative serum CA-125 establishes a baseline value that may be useful when interpreting the patient's subsequent CA-125 levels during further treatment.

Second, our multivariable survival analysis revealed that, together with stage of disease, preoperative serum CA-125 was a significant independent predictor of survival. In contrast to others[4], our study

shows that lymphovascular invasion, myometrial invasion and tumor diameter are not associated with survival. These findings indicate that serum CA-125 may be a useful tool for prognostic purposes and disease assessment already in the preoperative setting, and could be helpful in counseling and informing patients about their prognosis. Furthermore, this could be relevant with regards to the choice of the appropriate surgeon to operate UPSC patients. In our participating hospitals UPSC patients have not always been operated by a gynecologic oncologist.[29] As a result, UPSC patients possibly did not receive optimal treatment for their disease. Comprehensive staging with maximum cytoreduction has been associated with a significant survival benefit in UPSC patients.[11,30,31] Although to our opinion all UPSC patients should be comprehensively staged, preoperative serum CA-125 may help to triage a subgroup of women diagnosed with UPSC who need radical debulking surgery. Finally, preoperative serum CA-125 could possibly be helpful in selecting appropriate UPSC patients to investigate a novel treatment approach using neoadjuvant chemotherapy (NACT). UPSC commonly presents at an advanced stage of disease and despite the introduction of more aggressive surgical approaches and the use of adjuvant combination chemotherapy, the prognosis of UPSC has been dismal with a 5-year survival <50% for advanced stage (Stage III-IV) patients. At present, data are accumulating using NACT in advanced stages of epithelial ovarian cancer, with 3-4 cycles of NACT prior to initial surgery, followed by additional adjuvant chemotherapy. This regimen reduces tumor burden, permitting less aggressive primary surgery. As a result, besides similar or even improved survival rates, associated postoperative complications and the requirement of intensive care unit admission and hospital stay are both reduced. Recently, also NACT prior to surgery of UPSC patients was investigated in a limited number of studies with promising results: reduction of disease prior to surgery was correlated with better survival outcome and improved quality of life by reduction of complications.[32-33] Although further research will be necessary to validate the possibly beneficial role of NACT prior to primary surgery in the treatment of UPSC patients, preoperative serum CA-125 could possibly help in the triage selecting those UPSC patients who benefit most, since patients with elevated preoperative serum CA-125 most likely have advanced stage disease with a poor prognosis. To note, elevated preoperative CA-125 had a positive predictive value of 86% for the presence of extra-uterine disease.

We are aware UPSC patients were included over a long period of time (i.e. 18 years), in which treatment modalities have changed (e.g. comprehensive staging and adjuvant chemotherapy), which could have impacted on survival. To assess whether time of treatment had an influence on the outcome, we divided the UPSC population into two groups: group 1 were patients from 1992-2000 (*N* = 32) and group 2 were patients from 2001-2009 (*N* = 34). Survival of the total group or by stage in group 1 was compared to survival of the total group or by stage in group 2. We found no difference in survival (data not shown).

The present study has an important limitation being retrospective. Not all included UPSC patients were radically debulked or comprehensively staged, for reasons like massive spread of disease, morbid obesity or medical co-morbidities. However, most patients without proper comprehensive

staging procedure already had obvious disseminated disease based on macroscopic tumor deposits, bone or lung metastases, or peritoneal disease. Furthermore, we found a relatively short median time of follow-up, inherent to the highly aggressive nature of UPSC and caused by the high number of stage IV UPSC patients included in our study. We also compared the groups of UPSC patients with (*N* = 66) and without (*N* = 49) preoperative CA-125 measurement and found no significant differences: all variables were equally distributed among the two groups (data not shown). The strengths of this multicentre study are that it comprises patients of five different institutions, with histology confirmed by a dedicated expert gyneco-pathologist. To date, it represents one of the largest series on patients with this rare histology in literature.

In conclusion, serum CA-125 could have potential clinical application in the management of UPSC patients. Serum CA-125 elevation is predictive for the presence of extra-uterine disease already in a preoperative setting. Furthermore, it can be used for prognostication since preoperative serum CA-125 is a significant independent risk factor for survival. However, to warrant true clinical significance, a prospective study should be performed to evaluate serum CA-125 marker performance in UPSC patients.

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Chapter 4

Cervical cytology in serous and endometrioid endometrial cancer

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ABSTRACT

Objective

To determine the frequency of abnormal cervical cytology in preoperative cervical cytology of patients diagnosed with uterine papillary serous carcinoma (UPSC) and endometrioid endometrial carcinoma (EEC). Additionally, associations between abnormal cervical cytology and clinicopathological factors were evaluated.

Methods

Multicentre study in which EEC patients diagnosed at two hospitals from 1999-2009, and UPSC patients diagnosed at five hospitals from 1992-2009, were evaluated. Revision of the histological slides was performed systematically and independently by three gyneco-pathologists. Cervical cytology within six months before histopathological diagnosis of endometrial carcinoma was available for 267 EEC and 80 UPSC patients. Cervical cytology with atypical, malignant, or normal endometrial cells in post-menopausal women was considered as abnormal cytology, specific for endometrial pathology.

Results

Abnormal cervical cytology was found in 87.5% of UPSC patients, compared to 37.8% in EEC patients.

In UPSC, abnormal cytology was associated with extra-uterine spread of disease ($p = 0.043$). In EEC, abnormal cytology was associated with cervical involvement ($p = 0.034$). In both EEC and UPSC patients abnormal cervical cytology was not associated with survival.

Conclusion

Abnormal cervical cytology was more frequently found in UPSC patients. It was associated with extra-uterine disease in UPSC patients, and with cervical involvement in EEC patients. More prospective research should be performed to assess the true clinical value of preoperative cervical cytology in endometrial cancer patients.

INTRODUCTION

Endometrial cancer is the most frequent malignancy of the female genital tract in the western world.[1] The majority of patients with endometrial cancer are diagnosed with endometrioid endometrial carcinoma (EEC), typically presenting at an early stage with an excellent prognosis; five-year overall survival rates of 70-80% have been reported.[2,3] In contrast, uterine papillary serous carcinoma (UPSC) represents only 10% of endometrial cancers, but accounts for up to 39% of all endometrial cancer deaths. UPSC is therefore recognized as an aggressive tumor.[4] Unlike its EEC counterpart, UPSC commonly presents with advanced stage disease and poor prognosis, indicated by a five-year survival rate of only 18-45%.[4,5]

The treatment of endometrial cancer is primarily based on surgery, consisting of hysterectomy and bilateral salpingo-oophorectomy (BSO). There is no worldwide consensus whether pelvic and/or para-aortic lymphadenectomy should be performed as part of the staging procedure.[6,7] For EEC patients the extensiveness of surgery depends on the presence of risk factors for metastatic disease, like high tumor grade, deep myometrial invasion, and cervical involvement.[7] However, preoperative assessment of these factors remains a challenge. After histopathologic examination, only 8-13% of the EEC patients have cervical involvement, and about 7% will have extra-uterine disease at the time of diagnosis.[8,9] In contrast, in UPSC patients 55-87% have microscopic or macroscopic metastases outside the uterus at the time of diagnosis.[10,11] For these patients, debulking surgery and comprehensive surgical staging (including hysterectomy, BSO, bilateral pelvic lymph node dissection with para-aortic lymph node sampling and omental biopsy or omentectomy) have been suggested to more reliably determine stage of disease, to guide adjuvant treatment, and to improve survival.[12-14]

In asymptomatic women, cervical cytology appeared to be a poor screening tool for endometrial carcinoma because of its low sensitivity.[15,16] However, when normal or atypical endometrial cells are found in cervical cytology of postmenopausal women, it is predictive for endometrial pathology.[15,17,18] Furthermore, cervical cytology has shown to be of additional value for the prediction of cervical stroma involvement and lymph node metastases.[19-21] In addition, in patients with endometrial cancer, cervical cytology with atypical or malignant endometrial cells was associated with advanced stage of disease, high tumor grade and deep myometrial invasion.[22-26]

In patients with endometrial cancer, the frequency of atypical or malignant endometrial cells in preoperative cervical cytology varies from 31-50%.[22,24,25] It has been reported though, that in UPSC patients cervical cytology is more likely to contain atypical or malignant endometrial cells.[27] However, cervical cytology has only been investigated in cohorts with limited numbers of UPSC patients, and evidence for associations with poor prognostic factors in UPSC patients is lacking.[25,27-30]

The aim of this study was to evaluate the presence of shedded atypical endometrial cells in cervical cytology during the diagnostic and/or preoperative workup of patients with UPSC as compared to patients with EEC. Possible associations between abnormal cervical cytology and clinicopathological variables were studied in both cohorts. Furthermore, we evaluated whether the presence of atypical endometrial cells in preoperative cervical cytology has prognostic significance for survival in UPSC and EEC patients.

MATERIALS & METHODS

Patient selection

The nationwide network and registry of histo- and cytopathology in the Netherlands (Pathologisch Anatomisch Landelijk Geautomatiseerd Archief: PALGA)[31] was used to search for all patients with primary EEC, diagnosed and treated at the Radboud University Nijmegen Medical Centre or the Canisius Wilhelmina Hospital in the period between January 1999 and December 2009. Furthermore, this network was used to search for all patients diagnosed with UPSC in the same two hospitals and three additional hospitals (Rijnstate Hospital Arnhem, Gelderse Vallei Hospital Ede, and Maas Hospital Boxmeer) from January 1992 till December 2009. Patients were excluded in case of a second primary malignancy. Pathological, medical and operative records of all patients were retrieved from the hospitals involved.

All histopathological slides from surgery specimens were reviewed systematically by three expert pathologists (SZ, AW and JB). Review included tumor histology, tumor grade, depth of myometrial invasion, and the presence of lymphovascular space invasion (LVSI). Per definition, UPSC was considered to be grade 3 carcinoma.[32,33] Patients with UPSC were included when the carcinoma comprised at least 10% serous histology according to previously published criteria.[32,34] The included UPSC cases were defined as pure UPSC, with the serous component comprising >75% of the total tumor, and mixed UPSC, with the serous component comprising 10-75% of the tumor.

Revised UPSC and EEC patients were included when cervical cytology was taken for the diagnostic and/or preoperative workup because of clinical suspicion for malignancy, within a time frame of 6 months prior to histopathological diagnosis. To note, all included patients had cervical cytology taken before endometrial biopsy or dilatation and curettage. In case multiple cervical smears were taken during this 6 months time interval, the smear with the most severe diagnosis was used for our analyses.

Cervical Cytology

PALGA was used to retrieve the complete cervical cytology history of each EEC and UPSC patient. This database has nationwide coverage from 1991 onwards, showing all surgical specimens and cervical cytology ever taken from each patient, both by the general practitioner and medical specialist.[31] Within our study period, cervical cytology was obtained using both conventional cytology and the more recently introduced liquid based cytology.[35] Liquid based cytology was introduced between 1996 and 2003 at the different pathology laboratories. Cervical cytology was screened and classified by cytotechnologists and approved by a pathologist according to the CISOE-A classification system, of which results are easily translatable to the various Bethesda 2001 subcategories.[36] CISOE-A explicitly specifies the presence of normal and abnormal endometrial cells. Cervical cytology was classified as normal if there were no endometrial cells present. Atypical or malignant endometrial cells that were diagnosed in diagnostic or preoperative cervical cytology was considered as an abnormal cytological result, indicating endometrial pathology. Cervical cytology showing normal

endometrial cells was considered abnormal only in postmenopausal women. To note, atypical squamous or atypical glandular / endocervical cells were not considered as abnormal cytology.

Because of the retrospective study design, patients were staged according to the 1988 FIGO surgical staging system.[37] All patients underwent primary surgical treatment, except for four UPSC patients because of serious comorbidity ($N = 3$) or because the patient refused treatment ($N = 1$). These four patients remained to be included in our study to investigate the frequency of abnormal endometrial cells in preoperative cervical cytology and their relationship with various clinicopathological variables. However, these four UPSC patients did not receive optimal surgical and/or adjuvant treatment and were therefore excluded from further analyses on survival. Clinicopathological data were collected regarding age, body mass index (BMI), FIGO stage, peritoneal cytology, lymph node metastases, cervical involvement, extra-uterine disease, histology, and tumor diameter. Extra-uterine spread of disease was defined as cervical involvement, nodal involvement, positive peritoneal cytology, and/or disease at any other site outside the uterus.

Statistical analysis

To analyze the correlation between different clinicopathological variables with abnormal cervical cytology, univariable analyses were performed, with χ^2 or Fisher's exact test analyses for categorical variables, independent T-test for continuous variables, and univariable logistic regression analyses when appropriate. Furthermore, to examine the effects of various clinicopathogical variables on progression free survival (PFS), univariable analyses were performed using the Cox proportional hazard method. PFS was defined as the time in months from initial surgery to the date of recurrence. In case of no recurrence, the date of last contact or death was used for censoring. All statistical analyses were performed using SPSS statistical software for windows, version 18.0 (SPSS, Inc, Chicago, IL). $P < 0.05$ was considered statistically significant.

RESULTS

Records of 141 UPSC patients and 353 EEC patients were retrieved from the hospitals involved. In the UPSC-group, 26 patients were excluded because the serous component within the tumor comprised less than 10%, whereas 19 EEC patients were excluded because of (partly) non-endometrioid histology. Furthermore, from 35 UPSC patients and 67 EEC patients cervical cytology was either not taken for diagnostic/preoperative workup within 6 months prior to diagnosis, or unsatisfactory for diagnosis, and these patients were excluded. Thus, a total of 80 UPSC and 267 EEC patients comprised our study population.

Demographic and histopathological characteristics of the UPSC and EEC patients are presented in Table 1. For UPSC patients, the median age at diagnosis was 72 years (range from 47 to 86), whereas the median age at diagnosis for EEC was 63 years (range from 38 to 92). Forty-three (53.7%) UPSC patients were diagnosed with advanced stage (III-IV) disease, compared to 30 (11.2%) EEC patients.

In 52 (65.0%) UPSC patients extra-uterine spread of disease was found, compared to 52 (19.5%) of the patients with EEC. UPSC patients, compared to EEC patients, more often had LVSI (46.1% and 21.1% respectively), cervical involvement (40.3% and 12.4% respectively) deep myometrial invasion (52.0% and 40.4% respectively), and a larger median tumor diameter (4.0 and 2.5 respectively). In 36 UPSC patients (45%) lymphadenectomy was performed of whom 36.1% had positive pelvic or para-aortic lymph nodes. To note, among UPSC patients without proper lymph node sampling, the majority had obvious disseminated disease based on macroscopic tumor deposits, bone or lung metastases or peritoneal disease. In EEC patients, lymph node sampling was omitted in cases without clinical suspicion of FIGO stage II or more, as recommended by the Dutch guidelines for endometrioid endometrial cancer treatment. Thirty-five EEC patients (13.1%) underwent lymphadenectomy of whom 8.6% had positive lymph nodes. The median follow-up time for UPSC patients was 19 months (range 1 - 163 months) and 47 months (range 0 - 126 months) for EEC patients. Thirty-seven UPSC patients (48.7%) and 34 EEC patients (14.8%) had recurrence of disease, resulting in a five-year PFS of 40.2% and 90.8% respectively.

Table 1: Clinical and pathological characteristics of patients with UPSC and EEC.

	UPSC patients (*N* = 80)		**EEC patients (*N* = 267)**	
Variables	**Median (range) / *N* (%)**		**Median (range) / *N* (%)**	
Age at diagnosis (years)	72 (47-86)		63 (38-92)	
BMI (kg/m^2)	27.0 (18.0-41.0)		28.3 (18.4-53.6)	
FIGO Stage				
I	28	(35.0%)	215	(80.5%)
II	9	(11.3%)	22	(8.2%)
III	21	(26.3%)	18	(6.7%)
IV	22	(27.4%)	12	(4.5%)
Peritoneal Cytology				
Negative	42	(63.6%)	189	(91.7%)
Positive	24	(36.4%)	17	(8.3%)
Not sampled	14		61	
Para-aortic and/or Pelvic Lymph nodes				
Negative	23	(63.9%)	32	(91.4%)
Positive	13	(36.1%)	3	(8.6%)
Not sampled[a]	44		232	
Extra-uterine disease				
No	28	(35.0%)	215	(80.5%)
Yes	52	(65.0%)	52	(19.5%)

Table 1 (continued)

	UPSC patients (*N* = 80)		**EEC patients (*N* = 267)**	
Variables	**Median (range) / *N* (%)**		**Median (range) / *N* (%)**	
Cervical involvement				
No	46	(59.7%)	234	(87.6%)
Yes	31	(40.3%)	33	(12.4%)
Unknown	3		0	
LVSI				
No	41	(53.9%)	202	(78.9%)
Yes	35	(46.1%)	54	(21.1%)
Unknown	4		11	
Histology				
Pure histology	62	(77.5%)	NA[$]	
Mixed histology	18	(22.5%)		
Tumor Grade				
1	0	(0.0%)	115	(43.0%)
2	0	(0.0%)	110	(41.2%)
3	80	(100.0%)	42	(15.7%)
Myometrial invasion				
≤1/2 myometrium	36	(48.0%)	159	(59.6%)
>1/2 myometrium	39	(52.0%)	108	(40.4%)
Unknown	5		0	
Diameter of tumor (cm)	4.0	(0.5 - 10.0)	2.5	(0.2-8.0)
Median time of follow-up (months)	19	(1-163)	47	(0-126)

[a]Lymph node status assigned only by inspection/palpation at laparotomy or from imaging; FIGO: International Federation of Obstetrics and Gynecologists; LVSI: lymphovascular space invasion; UPSC: uterine papillary serous carcinoma; EEC: endometrioid endometrial carcinoma. [$]Not available: the variable 'histology' is a constant in EEC patients.

Preoperative cervical cytology

The cervical cytology findings in both UPSC and EEC patients are listed in Table 2. The median time interval between initial cervical cytology and final histopathological diagnosis for endometrial carcinoma was 1 month (range 0-6 months) for UPSC patients and 1.5 month (range 0-6 months) for EEC patients. In the group with 80 UPSC patients, 20 patients (25.0%) had atypical endometrial cells and 50 (62.5%) had malignant endometrial cells in their cervical cytology. Seven patients (8.8%) had normal cervical cytology preoperatively, and three patients (3.7%) had abnormal cervical cytology not specific for endometrial pathology: two cases with atypical glandular / endocervical epithelial cells and one case with atypical squamous epithelial cells. In total, 70 UPSC patients (87.5%) had abnormal cervical cytology, specific for endometrial pathology preoperatively (Table 2).

Within the group of 267 EEC patients, in 48 patients (18.0%) atypical endometrial cells were found in cervical cytology. In four patients (1.5%) normal endometrial cells were found, these four patients were postmenopausal. Forty-nine patients (18.4%) had malignant endometrial cells in their preoperative cervical cytology. In 163 EEC patients (61.0%) normal cervical cytology was found preoperatively, and three patients (1.1%) had abnormal cytology findings not specific for endometrial pathology: two with atypical squamous cells, and one with atypical glandular / endocervical cells. In total 101 EEC patients (37.8%) had abnormal cervical cytology, specific for endometrial pathology, prior to their diagnosis.

Table 2: Preoperative cervical cytology findings within six months prior to diagnosis of UPSC or EEC.

	UPSC patients (*N* = 80)		EEC patients (*N* = 267)	
Pathology in cervical cytology	***N* (%)**		***N* (%)**	
Normal endometrial cells in smear	0	(0%)	4	(1.5%)
Atypical endometrial cells in smear	20	(25.0%)	48	(18.0%)
Malignant endometrial cells in smear	50	(62.5%)	49	(18.3%)
Total abnormal cervical cytology	**70**	**(87.5%)**	**101**	**(37.8%)**
Cervical cytology without pathology	7	(8.8%)	163	(61.1%)
Other[#] pathology in smear	3	(3.7%)	3	(1.1%)
Total normal cervical cytology	**10**	**(12.5%)**	**166**	**(62.2%)**

UPSC: uterine papillary serous carcinoma; EEC: endometrioid endometrial carcinoma; [#]Atypical squamous epithelial cells, or atypical glandular / endocervical epithelial cells.

Associations of abnormal cervical cytology with clinicopathological findings

In UPSC patients, only extra-uterine spread of disease ($p = 0.043$) was significantly associated with an increased frequency of abnormal cervical cytology (Table 3). Using univariable logistic regression, extra-uterine disease remained the only factor associated with abnormal cervical cytology (OR 5.11, 95% CI 1.02 – 28.36; data not shown). Advanced stage of disease was not associated with abnormal cervical cytology; 23 of 28 UPSC patients (82.1%) with stage I disease already had abnormal cervical cytology. In addition, patients lacking poor prognostic factors, like negative peritoneal cytology, absence of lymph node metastases, or no cervical involvement, had abnormal cervical cytology in 37 of 40 (92.5%), 20 of 21 (95.2%), and 41 of 46 (89.1%) cases, respectively. There was no significant difference in frequency of abnormal cervical cytology among pure UPSC and mixed UPSC patients. Other well known prognostic factors such as myometrial invasion, LVSI, and tumor diameter, were also not associated with abnormal cervical cytology in UPSC patients.

In EEC patients abnormal cervical cytology was significantly associated with cervical involvement ($p = 0.034$). All other clinicopathological variables were not associated with abnormal cervical cytology in EEC patients (Table 3). Cervical involvement was the only variable associated with abnormal cervical cytology in EEC patients using univariable logistic regression (OR 1.61, 95% CI 1.14 – 4.88; data not shown).

Table 3: Associations preoperative cervical cytology findings with clinicopathological variables in patients with UPSC and EEC.

	Cervical cytology in UPSC patients[‡]			Cervical cytology in EEC patients[‡]		
Variable	Normal	Abnormal	*p*-value	Normal	Abnormal	*p*-value
Mean Age (years)	75.7	70.5	0.140[#]	64.6	63.4	0.270[#]
Mean BMI (kg/m^2)	26.7	27.0	0.872[#]	28.9	30.5	0.092[#]
FIGO Stage						
I	5 (71.4%)	23 (32.8%)	0.151[∞]	139 (83.7%)	76 (75.3%)	0.392
II	0 (0.0%)	7 (10.0%)		12 (7.3%)	10 (9.9%)	
III	0 (0.0%)	20 (28.6%)		9 (5.4%)	9 (8.9%)	
IV	2 (28.6%)	20 (28.6%)		6 (3.6%)	6 (5.9%)	
Peritoneal Cytology						
Negative	3 (60.0%)	37 (62.7%)	0.904[∞]	125 (92.6%)	64 (90.1%)	0.543
Positive	2 (40.0%)	22 (37.3%)		10 (7.4%)	7 (9.9%)	
Para-aortic and/or Pelvic Lymph nodes						
Negative	1 (50.0%)	20 (62.5%)	0.724[∞]	20 (100.0%)	12 (80.0%)	0.070[∞]
Positive	1 (50.0%)	12 (37.5%)		0 (0.0%)	3 (20.0%)	
Extra-uterine spread						
No	5 (71.4%)	23 (32.9%)	**0.043**[∞]	139 (83.7%)	76 (75.2%)	0.089[∞]
Yes	2 (28.6%)	47 (67.1%)		27 (16.3%)	25 (24.8%)	
Cervical involvement						
No	5 (83.3%)	41 (60.3%)	0.265[∞]	151 (91.0%)	83 (82.2%)	**0.034**
Yes	1 (16.7%)	27 (39.7%)		15 (9.0%)	18 (17.8%)	
LVSI						
No	3 (42.9%)	36 (54.5%)	0.556[∞]	129 (81.6%)	73 (74.5%)	0.173
Yes	4 (57.1%)	30 (45.5%)		29 (18.4%)	25 (25.5%)	
Histology						
Pure histology	5 (71.4%)	56 (80.0%)	0.594[∞]	166 (100.0%)	101 (100.0%)	NA[$]
Mixed histology	2 (28.6%)	14 (20.0%)		0 (0.0%)	0 (0.0%)	
Tumor Grade						
1	0 (0.0%)	0 (0.0%)	NA[*]	78 (47.0%)	37 (36.6%)	0.274
2	0 (0.0%)	0 (0.0%)		65 (39.2%)	45 (44.6%)	
3	7 (100.0%)	70 (100.0%)		23 (13.8%)	19 (18.8%)	
Myometrial invasion						
≤1/2 myometrium	2 (33.3%)	32 (48.5%)	0.477[∞]	98 (59.0%)	61 (60.4%)	0.826
>1/2 myometrium	4 (66.7%)	34 (51.5%)		68 (41.0%)	40 (39.6%)	
Mean Diameter of tumor (cm)	3.1	4.0	0.343[#]	2.9	2.6	0.236[#]

[‡]Adjusted for abnormal cytology specific for endometrial pathology; [#]Independent T-test was used for continuous variables; [∞]Fisher's exact test; [*]Not available: the variable 'tumor grade' is a constant in UPSC patients. [$]Not available: the variable 'histology' is a constant in EEC patients. LVSI: lymphovascular space invasion; UPSC: uterine papillary serous carcinoma; EEC: endometrioid endometrial carcinoma.

Survival analysis

In a Cox proportional hazard model clinicopathological variables including preoperative cervical cytology were analyzed for their association with progression free survival (PFS). In univariable analyses in UPSC patients, FIGO stage, extra-uterine disease, lymph node metastases, and LVSI were significantly associated with PFS (Table 4). Abnormal cervical cytology was not associated with PFS in UPSC patients. In addition, no associations were found between PFS and age, BMI, cervical involvement, myometrial invasion, tumor diameter, or the composition of the tumor (pure or mixed UPSC). In EEC patients, FIGO stage, tumor grade, extra-uterine disease, cervical involvement, LVSI, myometrial invasion, and tumor diameter were significantly associated with PFS (Table 4). However, also in EEC patients abnormal cervical cytology specific for endometrial pathology was not associated with PFS.

Table 4: Crude hazard ratios (HR) with 95% confidence interval (CI) of Progression Free Survival (PFS) by clinicopathological variable in UPSC and EEC patients, using univariable Cox regression.

	PFS - UPSC		PFS - EEC	
Variable	***N***	**HR (95% CI)**	***N***	**HR (95% CI)**
Age at diagnosis (years)	76	1.00 (0.96 – 1.03)	283	1.03 (0.99 - 1.06)
BMI (kg/m^2)	68	0.93 (0.86 – 1.01)	242	0.99 (0.93-1.05)
FIGO Stage				
I – II	37	1.00 (reference)	251	1.00 (reference)
III – IV	39	**6.87 (3.22 – 14.67)**	32	**9.66 (4.91 - 19.01)**
Histology				
Pure	58	1.00 (reference)		NA[$]
Mixed	18	0.79 (0.36 – 1.73)		
Tumor Grade				
Low (grade 1 - 2)		NA[*]	242	1.00 (reference)
High (grade 3)			41	**5.82 (2.95 - 11.48)**
Extra-uterine disease				
No	28	1.00 (reference)	212	1.00 (reference)
Yes	48	**6.67 (2.75 – 16.16)**	51	**6.75 (3.39 - 13.43)**
Cervical involvement				
No	46	1.00 (reference)	230	1.00 (reference)
Yes	29	1.84 (0.95 – 3.55)	33	**3.29 (1.53 - 7.10)**
Para-aortic and/or Pelvic Lymph nodes				
Negative	21	1.00 (reference)	32	NA[#]
Positive	12	**3.96 (1.40 – 11.20)**	3	NA[#]

Table 4 (continued)

	PFS - UPSC		PFS - EEC	
Variable	***N***	**HR (95% CI)**	***N***	**HR (95% CI)**
LVSI				
No	41	1.00 (reference)	219	1.00 (reference)
Yes	35	**2.56 (1.34 – 4.92)**	53	**4.57 (2.27 - 9.18)**
Myometrial invasion				
≤1/2 myometrial	36	1.00 (reference)	172	1.00 (reference)
>1/2 myometrial	39	1.76 (0.92 – 3.37)	111	**2.42 (1.22 - 4.78)**
Maximum diameter tumor	63	1.13 (0.95 – 1.35)	103	**1.05 (1.01 - 1.10)**
Cervical cytology				
Normal	10	1.00 (reference)	180	1.00 (reference)
Abnormal	66	1.73 (0.61 – 4.89)	103	1.41 (0.72 - 2.77)

[*]Not available: the variable 'tumor grade' is a constant in UPSC patients. [$]Not available: the variable 'histology' is a constant in EEC patients. LVSI: lymphovascular space invasion; UPSC: uterine papillary serous carcinoma; EEC: endometrioid endometrial carcinoma. Not available: the number of EEC patients with proper lymph node sampling was insufficient.

DISCUSSION

In this study a high frequency of abnormal cervical cytology was found in UPSC patients compared to EEC patients (87.5% and 37.8% respectively). In UPSC patients, abnormal cervical cytology was significantly associated with extra-uterine disease, whereas in EEC patients an association was found with cervical involvement. Abnormal cervical cytology specific for endometrial pathology was not associated with survival in either UPSC or EEC patients.

The possible prognostic and diagnostic role of cervical cytology in patients with the suspicion of endometrial cancer has gained little attention so far. It was shown that cervical cytology may provide additional preoperative diagnostic information when normal, suspicious or malignant endometrial cells are detected. The presence of atypical endometrial cells has a significant correlation with the presence of endometrial cancer.[15,17,18,39] In addition, in endometrial cancer patients abnormal cervical cytology has been associated with unfavorable prognostic, clinical, and pathological parameters.[19,20,22,24,25,38] However, in none of these studies a difference was made between patients with EEC and UPSC.

The most striking finding in this study was the very high frequency of abnormal cervical cytology (87.5%) in UPSC patients relative to EEC patients. At present, there have only been a few studies with a limited number of patients on cervical cytology in UPSC patients, and frequencies of abnormal cervical cytology among UPSC patients have varied from 72-88%.[25,27-30] It has been suggested previously that cervical cytology of UPSC patients is more likely to contain suspicious or malignant endometrial cells, probably due to the papillary architecture of the tumor and the propensity to exfoliate.[38,40] Although the molecular biology to account for this observation has not been

thoroughly investigated, we and others propose there is a relation with change of expression of cell adhesion molecules such as CD44, integrin, e-cadherin, β-catenin and L1-CAM.[39-41] In addition, involvement of the endocervix by the serous uterine tumor is more prevalent when compared to EEC and hence could explain the high rate of positive cervical cytology.[40,42]

We showed that in UPSC patients extra-uterine disease was significantly associated with abnormal cervical cytology. Markedly, UPSC patients who lacked poor prognostic factors still had abnormal cervical cytology in most cases. This was illustrated by patients with negative peritoneal cytology or absence of lymph node metastases who had abnormal cervical cytology in 92.5% and 95.2% of the cases respectively. In EEC patients, we found an association of abnormal cervical cytology with cervical involvement. The prognostic impact of other clinicopathologic factors was in concordance with literature.[1,17] We did not find a prognostic impact of BMI in our analyses, probably due to our separate analyses of the EEC and UPSC cohort. Furthermore, the number of EEC patients might not be sufficient to find a prognostic impact of BMI.

Investigators have studied whether preoperative cervical cytology is an independent prognostic factor for survival in endometrial cancer patients. Although in one study by Fukuda and co-workers an association was found in univariable analyses[24], cervical cytology never was an independent prognosticator for survival. In concordance, we found no association between cervical cytology and progression free survival in both UPSC and EEC patients independently. The fact that abnormal cervical cytology was associated with extra-uterine disease but not with PFS in UPSC patients may be explained by the very large number of UPSC patients with advanced stage of disease with a dismal prognosis.

It was shown by some investigators that preoperative cervical cytology with atypical glandular / endocervical cells, especially in patients over 50 years of age, was associated with endometrial pathology in 5-25% of cases.[43,44] In our UPSC cohort two patients had atypical glandular cells, and in the EEC cohort one patient had atypical glandular cells. When these smears were considered as abnormal cytology, results of the analyses were not different. In the UPSC group extra-uterine spread of disease remained the only variable significantly associated with an increased frequency of abnormal cervical cytology (OR 5.33, 95% CI 1.12 – 29.54; data not shown), whereas cervical involvement remained the only variable significantly associated with abnormal cervical cytology in EEC patients (OR 5.67 95% CI 1.07-30.09; data not shown). Furthermore, abnormal cervical cytology was still not associated with PFS in both cohorts (data not shown).

It is of great importance to identify UPSC already in the preoperative setting, because of UPSC's aggressive behavior and different surgical and adjuvant treatment approach compared to its EEC counterpart, including radical debulking surgery, comprehensive staging and chemoradiation therapy.[14,45] Biopsy of the endometrium using either outpatient biopsy techniques or dilatation and curettage has been the standard of care procedure to obtain a preoperative histopathological diagnosis.[6] However, diagnosis of the histological type made on these biopsies or curettage specimen can be challenging, with up to 20% of the diagnoses of histological type being changed

after surgery.[46] Preoperative cervical cytology might give an indication to suspect a more aggressive uterine tumor.

There are some limitations to this study, being retrospective as most important issue. In only a small portion of included EEC patients lymphadenectomy or lymph node sampling was performed, and not all UPSC patients were radically debulked and comprehensively staged for reasons like massive spread of disease, morbid obesity or medical co-morbidities. In addition, there was a change from conventional to liquid based cytology at different time points in the different laboratories involved. The sensitivity for the detection of endometrial cancer appears to be higher when liquid based cytology is used compared to conventional techniques.[47] However, we cannot comment on the difference in detection rate in the current study, since data on the cytology method are not available for all individual patients. Furthermore, we found a difference in median time of follow up between the EEC and UPSC cohort. However, this difference can be explained by the highly aggressive nature of UPSC, with its high mortality rate already soon after clinical presentation, and poor five-year overall survival rate compared to EEC patients. This multicentre study comprises patients of five different institutions, with UPSC and EEC histology confirmed by three dedicated gyneco-pathologists, and with a complete national coverage of cervical cytology history. Furthermore, in all included patients cervical cytology was taken before endometrial biopsy or dilatation & curettage, with a median time interval of 1-2 months prior to final histopathological diagnosis. To our knowledge this is the first study analyzing endometrial pathology in cervical cytology in two large cohorts of UPSC and EEC patients specifically.

In conclusion, abnormal cervical cytology was more frequently found in UPSC patients. It was associated with extra-uterine disease in UPSC patients and with cervical involvement in EEC patients. More prospective research should be performed to assess the true clinical value of preoperative cervical cytology in endometrial cancer patients.

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Chapter 5

Pure compared with mixed serous endometrial carcinoma: Two different entities?

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ABSTRACT

Objective:

To analyze whether mixed compared with pure uterine papillary serous carcinoma (UPSC) histology affects clinical outcome, and to assess UPSC for its association with the precursor lesion endometrial intraepithelial carcinoma (EIC).

Methods:

A multi-institution observational study on stage I – IV UPSC patients was performed. Histopathologic slides were reviewed by four expert pathologists, with determination of the percentage serous histology within each tumor. The pre-existent endometrium was evaluated for the presence of EIC.

Results:

We included 108 UPSC patients. Fifty-eight patients had mixed and 50 patients had pure UPSC histology. On multivariable analysis, advanced International Federation of Gynecology and Obstetrics (FIGO) stage (HR 3.15, 95%CI 1.57 – 6.32), mixed UPSC histology (HR 0.35, 95%CI 0.19 – 0.66), and lymphovascular space invasion (HR 2.10, 95%CI 1.07 – 4.16) were significantly associated with recurrence. FIGO stage (HR 4.67, 95%CI 2.25 – 9.70) and mixed UPSC histology (HR 0.39, 95%CI 0.20 – 0.76) were significantly and independently associated with survival. EIC was identified in 83.9% of all cases, with no significant difference between mixed and pure UPSC patients. Atrophic or weakly proliferative endometrium was found in 90.7% of pure UPSC cases, whereas hyperplastic endometrium with atypia was more commonly found in 34.7% of patients with mixed UPSC ($p = 0.004$).

Conclusion:

Pure UPSC histology and FIGO stage are the most important risk factors for recurrence and survival in patients with UPSC. Adjusted for covariates, patients with pure UPSC had a 2.9 times greater risk for recurrence and a 2.6 times higher risk of death compared to patients with mixed UPSC. Furthermore, EIC was equally found among pure and mixed UPSC cases, whereas the nonneoplastic endometrium was atrophic or weakly proliferative in pure UPSC cases compared to more hyperplastic endometrium with atypia in mixed UPSC cases.

INTRODUCTION

Uterine papillary serous carcinoma (UPSC) is a highly aggressive subtype of endometrial cancer, disproportionally responsible for up to 39% of deaths due to endometrial cancer.[1,2] Typically, 55-87% of the UPSC patients have extra-uterine spread of disease at the time of diagnosis.[5,6]

UPSC often displays considerable morphologic heterogeneity, co-existing with at least one other subtype of uterine cancer, most often comprising an endometrioid or clear cell component.[7,8] However, it is largely unknown whether the percentage UPSC histology is predictive of recurrence rates or survival. It was proposed that patients with mixed UPSC had the same prognosis and risk for metastases as patients with pure UPSC.[7-14] In contrast, others described a favorable survival outcome for mixed UPSC patients compared to pure UPSC.[15,16] [17]

The natural history for endometrioid endometrial carcinoma has been fairly well characterized as progression from its precursor endometrial hyperplasia with atypia to an invasive carcinoma.[18,19] In contrast, the etiology of UPSC is not as well understood. Histopathologic studies suggest that the majority of uterine serous carcinomas develop from endometrial intraepithelial carcinoma (EIC), identified in 58-89% of uteri containing serous carcinoma.[8,20-22] However, the presence of EIC as precursor in mixed UPSC cases is largely unknown. It can be argued that the etiology and pathogenesis of mixed UPSC may differ from that of pure UPSC [23], possibly also resulting in a different clinical behavior.

The purpose of this study was to estimate whether mixed versus pure UPSC histology affects clinical outcome, and to identify clinicopathologic factors predictive for recurrence and survival in stage I-IV UPSC patients. Furthermore, we assessed the uninvolved endometrium in both pure and mixed UPSC cases for their association with the precursor lesion EIC or (atypical) hyperplasia or both.

PATIENTS & METHODS

Patient selection

For this observational study the Research Ethics Committee of the Radboud University Nijmegen Medical Centre declared that the study protocol is in accordance with the applicable rules concerning the review of research ethics committees and informed consent. The nationwide Netherlands database of histo- and cytopathology (PALGA)[24] was used to search for all patients with primary UPSC, diagnosed and treated at the Radboud University Nijmegen Medical Center, Rijnstate Hospital Arnhem, Canisius Wilhelmina Hospital Nijmegen, Gelderse Vallei Hospital Ede, and Maas Hospital Boxmeer, in the period between January 1992 till December 2009. Pathologic, medical and operative records were retrieved.

All histopathologic slides were independently reviewed by four expert pathologists (MB, JB, AW and SZ), on the basis of criteria outlined below. All pathologists were blinded to any clinical information. Review of the specimen included determination of tumor type and composition, depth of myometrial invasion, tumor diameter, and LVSI. Percentage histology was estimated by

each individual pathologist reviewing slides of the entire carcinoma using a low-magnification (50-100x) field. Individual scoring was assessed and discrepancies were resolved in a consensus meeting with the four pathologists. Each tumor was measured along the widest diameter, irrespective of histological component within mixed cases, with rounding at 0.5 centimeter. Furthermore, grade of nuclear atypia, presence of hobnail cells, psammoma bodies, and the number of mitotic figures were scored. The pre-existent and/or adjacent endometrium was evaluated for the presence of EIC. When available, the cervix, fallopian tubes, ovaries, lymph nodes and other sites were examined for metastatic disease. Per definition, UPSC was considered to be high-grade.[7,8]

Women were included in which the carcinoma comprised at least 10% serous histology in their respective uterine pathologic specimen after review, according to previously published criteria: "an epithelial subtype must be at least 10% of the total volume to designate the tumor mixed".[7,13] Patients were excluded in case of a second primary malignancy. In cases where tumor involved the ovaries, only those that did not form a discrete ovarian mass typical for a primary ovarian carcinoma were included. Cases in which the entire endometrium was replaced by invasive tumor were not excluded ($N = 15$).

The diagnosis UPSC was based on earlier described criteria.[7,8] In brief, the serous component of the carcinoma was characterized by a complex papillary growth pattern, although glandular and solid patterns may also occur, with numerous mitotic figures, psammoma bodies, fibrovascular micro-papillae and large, branching glands lined by papillary tufts composed predominantly of cuboidal or hobnail cells. Marked nuclear atypia should always be present.

The endometrium uninvolved by invasive tumor was classified as atrophic/weakly proliferative, hyperplastic with/without atypia, or EIC. Atrophic/weakly proliferative endometrium was defined as thin endometrium, composed of dense stroma in which there were widely spaced simple tubular glands lined by atrophic-appearing epithelium in which there were few mitotic figures, or by more columnar and pseudostratified mitotically inactive epithelium.[20,22]

Endometrial hyperplasia was characterized by an increase in glandular density relative to the adjacent endometrial stroma. Hyperplastic endometrium, when present, was classified as simple with or without atypia, or complex with or without atypia.[8,20]

EIC was characterized by the replacement of benign surface endometrial epithelium or endometrial glands by a layer of markedly atypical cells resembling UPSC, usually adjacent to atrophic/weakly proliferative epithelium.[20,21] The lesion can be very small and both focal or multifocal, and is often present in a polyp. Cytologically, the cells show marked nuclear membrane irregularities, mitotic figures including abnormal forms, and apoptotic bodies. By definition, EIC is not characterized by proliferation of glands as in complex hyperplasia with atypia. Both on the surface and in partially affected glands, there is a sharp demarcation between the morphologically malignant and atrophic/weakly proliferative epithelium.[20,22]

Stage of disease was assigned based on the 2009 International Federation of Gynecology and Obstetrics (FIGO) endometrial cancer criteria.[25] Surgical treatment with intent to achieve maximal

cytoreduction included total abdominal hysterectomy and bilateral salpingo-oophorectomy, with/without pelvic and para-aortic lymph node sampling or lymphadenectomy, and omentum sampling. In case of incomplete surgical staging, stage was assigned on the basis of available operative and pathology findings. UPSC histology was analyzed both as a continuous variable and categorically as mixed UPSC (10-90% serous component) versus pure UPSC (100% serous component). Subgroup analyses of patients with 10-40% and 50-90% UPSC histology were also performed. Clinicopathological data were collected regarding age, menopausal state, presenting symptom, FIGO stage, peritoneal cytology, lymph node metastases, and tumor diameter. Tumor diameter was analyzed as both a continuous and categorical variable (≤ 4.0cm versus > 4.0cm).

Patient demographic and histopathologic characteristics were compared between mixed UPSC and pure UPSC patients, tested for statistical significance using one-way ANOVA for continuous variables. For categorical variables the Pearson's chi-square (χ^2) test or Fisher's exact test was used when appropriate. Univariable proportional hazards models were used to examine the influence of various clinicopathological variables on recurrence and survival. Recurrence was defined as the first evidence of disease or death of disease after a six months disease-free interval after initial surgery. Length of overall survival (OS) was calculated from the date of initial surgery to the date of death or of last contact, whereas surviving patients were censored at the date of last contact. Progression free survival (PFS) was defined as the time in months from initial surgery to the date of recurrence. Multivariable proportional hazards models with a stepwise forward selection procedure were used to examine recurrence, OS, and PFS, while adjusting for demographic and tumor variables. Only variables with a p-value < 0.10 on univariable analyses were entered in the multivariable regression model. Furthermore, OS and PFS estimates were plotted utilizing the Kaplan-Meier method and compared between mixed-UPSC and pure UPSC using Log-rank statistics. We also performed sub-group analyses for FIGO stage (stage I-II compared to III-IV). All statistical analyses were performed using SPSS statistical software, version 18.0 for windows (SPSS, Inc, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Records of 141 patients were retrieved from the hospitals involved. Twenty-six patients were excluded because the serous component comprised <10% of the tumor. All patients underwent primary surgical treatment, except for seven UPSC patients because of serious comorbidity ($N = 5$) or because the patient refused treatment ($N = 2$). These seven patients were also excluded. Although UPSC is considered high-grade, 13 cases (12%) showed low-grade nuclear atypia. Serous carcinoma may show considerable cytologic variability throughout the tumor [26], and as the tumors with low-grade nuclear atypia further showed the typically serous architecture, we included these cases as true UPSC. A total of 108 UPSC patients met the inclusion criteria.

Patient demographic and clinical characteristics were analyzed among mixed UPSC and pure UPSC

cases (Table 1). Mean age at diagnosis was 70.9 and not significantly different among the two groups. For the total cohort, 48.1% had stage III-IV disease, and distribution of stage of disease was not significantly different among mixed versus pure UPSC cases. In addition, menopausal state, BMI, and main presenting symptom were not different between the groups. Furthermore, no significant differences in adjuvant treatment were found among mixed and pure UPSC patients, with 39.7% and 36.0% receiving no adjuvant treatment, respectively. Forty-seven percent of all patients were not fully comprehensively staged, though this was not significantly different among mixed and pure UPSC cases (46.6% and 48.0%, respectively). To note, sub-analyses using completely staged patients only revealed similar results among pure and mixed UPSC cases (data not shown). Mean follow-up for the total study cohort was 29 months. The mean follow-up period differed significantly among the sub-groups, with 34.7 months (median 24.0 months, range 1-163) for mixed UPSC versus 21.8 months (median 15.5 months, range 0-132) for pure UPSC cases ($p = 0.026$).

For the total UPSC cohort, per case a median number of 5 slides (range 2-20) of the uterine specimen were reviewed. Histopathologic tumor characteristics by sub-group are presented in Table 1. We found 50 patient cases (46.3%) with pure UPSC histology (100% serous component) whereas 58 cases (53.7%) had mixed UPSC histology (10-90% serous component). Of the 58 mixed UPSC cases, 45 (77.6%) had concurrent endometrioid adenocarcinoma, 8 patients (13.8%) had clear cell carcinoma, and 5 patients (8.6%) had a concurrent component of undifferentiated carcinoma. To note, the endometrioid component within mixed UPSC cases was most often low grade. In the mixed cases, the deepest areas of invasion were usually composed of UPSC histology, and 20 of 45 (44.4%) mixed endometrioid-UPSC cases had extra-uterine disease consisting predominantly of the serous component. Typically, the total UPSC cohort showed LVSI and deep myometrial invasion in 53 (49.1%) and 51 (47.7%) cases, respectively. Thirty-eight patients (35.2%) had both LVSI and deep myometrial invasion. Tumor diameter, carcinoma confined to an endometrial polyp, positive LVSI, and deep myometrial invasion were not significantly different among mixed versus pure UPSC cases. In addition, the serous component of mixed UPSC resembled the pure UPSC: marked nuclear atypia, the presence of psammoma bodies and/or hobnail cells, and the amount of mitotic figures did not differ significantly between the two sub-groups (Table 1).

In 15 cases, the serous carcinoma invaded all the endometrium available for review. In the remaining 93 patients, the precursor lesion EIC was identified within the uterine specimen in 78 cases (83.9%). EIC was equally distributed among the sub-groups: in 41 (83.7%) mixed UPSC cases EIC was identified, compared to 37 (84.1%) in pure UPSC cases (Table 1). Even in mixed carcinoma consisting of 10-40% UPSC histology, EIC was present in 9 of 10 cases (90.0%) (data not shown). In addition, EIC was most commonly identified adjacent to the serous carcinoma (79.5%), whereas it was present at distant sites only in 20.5%. EIC was multifocal in 52 of 78 cases (66.7%), with a median number of two EIC lesions per case (range 1 – 10). The distribution of EIC with respect to the serous carcinoma and the number of multifocal EIC lesions was not significantly different among the sub-groups (Table 1 and data not shown).

Table 1: Demographics, clinical and histopathological characteristics of pure versus mixed uterine papillary serous carcinoma patients ($N = 108$).

	Mixed UPSC ($N = 58$)		**Pure UPSC ($N = 50$)**		
Variables	***N* (%) / Mean (SD)**		***N* (%) / Mean (SD)**		***p*-value**
Mean age at diagnosis (years)	70.8	(+/- 9.1)	70.9	(+/- 8.7)	0.956[#]
Menopausal state					
Premenopausal	3	(5.2%)	2	(4.0%)	0.772
Postmenopausal	55	(94.8%)	48	(96.0%)	
BMI (kg/m^2)					
≤30	34	(72.3%)	33	(82.5%)	0.262
>30	13	(27.7%)	7	(17.5%)	
Main presenting symptom					
Blood loss	53	(91.4%)	44	(88.0%)	0.629
Abdominal pain	2	(3.4%)	4	(8.0%)	
Other	3	(5.2%)	2	(4.0%)	
FIGO stage					
I + II	33	(56.9%)	23	(46.0%)	0.258
III + IV	25	(43.1%)	27	(54.0%)	
Adjuvant treatment					
OBS	23	(39.7%)	18	(36.0%)	0.167
RT	25	(43.1%)	18	(36.0%)	
CT +/- RT	10	(17.2%)	14	(28.0%)	
Mean follow-up (months)	34.7	(+/- 32.6)	21.8	(+/- 25.3)	**0.026[#]**
Histology of other component					
Endometrioid	45	(77.6%)	NA[$]		
Clear cell	8	(13.8%)			
Undifferentiated	5	(8.6%)			
Nuclear atypia					
Slight	0	(0.0%)[*]	2	(3.8%)	0.578
Moderate	7	(12.5%)[*]	4	(7.7%)	
Severe	49	(87.5%)[*]	46	(88.5%)	
Hobnail-cells					
No	22	(37.9%)	13	(26.0%)	0.187
Yes	36	(62.1%)	37	(74.0%)	
Mitoses (per HPF)					
0-9	21	(36.2%)	18	(36.0%)	0.252
10-24	15	(25.9%)	7	(14.0%)	
>24	22	(37.9%)	25	(50.0%)	

Table 1 (continued)

	Mixed UPSC (*N* = 58)		Pure UPSC (*N* = 50)		
Variables	***N* (%) / Mean (SD)**		***N* (%) / Mean (SD)**		***p*-value**
Psammoma bodies					
No	44	(75.9%)	32	(64.0%)	0.178
Yes	14	(24.1%)	18	(36.0%)	
Mean tumor diameter (cm)	3.9	(+/- 2.7)	4.0	(+/- 1.7)	0.836[#]
Tumor in polyp					
No	38	(65.5%)	34	(68.0%)	0.785
Yes	20	(34.5%)	16	(32.0%)	
LVSI					
No	32	(55.2%)	23	(46.0%)	0.342
Yes	26	(44.8%)	27	(54.0%)	
Myometrial invasion					
≤1/2 myometrium	34	(59.6%)	22	(44.0%)	0.106
>1/2 myometrium	23	(40.4%)	28	(56.0%)	
EIC present[@]					
No	8	(16.3%)	7	(15.9%)	0.956
Yes	41	(83.7%)	37	(84.1%)	
EIC in relation to UPSC					
Adjacent only	21	(51.2%)	13	(35.1%)	0.159
At distance only	9	(22.0%)	7	(18.9%)	
Adjacent & at distance	11	(26.8%)	17	(46.0%)	
Uninvolved endometrium[€]					
Atrophic / weakly proliferative	32	(65.3%)	39	(90.7%)	**0.004**
Hyperplasia with atypia	17	(34.7%)	4	(9.3%)	

UPSC: uterine papillary serous carcinoma; SD: standard deviation; FIGO: International Federation of Obstetrics and Gynecologists; BMI: body mass index; OBS: observation only after surgery; RT: radiotherapy; CT +/- RT: Chemotherapy +/- radiotherapy; [$]NA: Not available, no other histological component present in pure UPSC cases; [*]Assessed in the serous component within mixed UPSC cases; LVSI: lymphovascular space invasion; HPF: high power field; EIC: endometrial intraepithelial carcinoma; [@] In 15 cases, the serous carcinoma invaded all the endometrium available for review; [€] In one case, the entire endometrium was replaced by EIC and UPSC; [#]One-way ANOVA was used for continuous variables.

The endometrium was atrophic/weakly proliferative in 71 of 92 cases (77.2%), and showed hyperplasia in 21 cases (22.8%). Six of these cases consisted of simple atypical hyperplasia and 15 cases consisted of complex atypical hyperplasia. The aspect of the uninvolved endometrium differed significantly among the two groups, with 4 of 43 (9.3%) pure UPSC cases having hyperplastic endometrium with atypia, of which two in an endometrial polyp without carcinoma, compared to 17 of 49 (34.7%) mixed UPSC cases (p = 0.004). In 15 of these 17 cases (88.2%) the hyperplastic endometrium with atypia was adjacent to an endometrioid component within mixed UPSC (data

not shown). Never hyperplastic endometrium with atypia was identified in close resemblance to UPSC or EIC. Atrophic or weakly proliferative endometrium was found in 90.7% of pure UPSC cases, compared to 65.3% in mixed UPSC cases. In 19 cases, both EIC and atypical hyperplasia were identified. In 14 of these 19 cases EIC was adjacent to UPSC, whereas hyperplastic endometrium with atypia was more commonly found at a distance from UPSC (data not shown). To note, 16 of 19 cases involved mixed UPSC. In a sub-group analysis, no association was found for higher BMI and endometrial hyperplasia with atypia (p = 0.195, data not shown).

Table 2: Crude and adjusted hazard ratios (HR) with 95% confidence interval (CI) for disease recurrence by clinicopathological variable of uterine papillary serous carcinoma patients (UPSC), using uni- and multivariable Cox regression with selection procedure.

	Univariable			Multivariable		
Variable	***N***	**HR (95% CI)**	***p*-value**	***N***	**HR (95% CI)**	***p*-value**
Age at diagnosis	82	1.00 (0.97 – 1.03)	0.980		NS[*]	
FIGO stage[#]						
I – II	54	1.00 (reference)	< 0.001	44	1.00 (reference)	0.001
III – IV	28	**3.47 (1.87 – 6.46)**		26	**3.15 (1.57 – 6.32)**	
Histology[#]						
Pure UPSC	32	1.00 (reference)	0.009	26	1.00 (reference)	0.001
Mixed UPSC	50	**0.44 (0.24 – 0.82)**		44	**0.35 (0.19 – 0.66)**	
LVSI[#]						
No	50	1.00 (reference)	0.001	42	1.00 (reference)	0.032
Yes	32	**2.89 (1.55 – 5.39)**		28	**2.10 (1.07 – 4.16)**	
Myometrial invasion						
≤1/2 myometrial	48	1.00 (reference)	0.162		NS[*]	
>1/2 myometrial	34	1.55 (0.84 – 2.86)				
Tumor diameter[#]	70	1.16 (0.99 – 1.36)	0.074		NS[*]	
Uninvolved endometrium						
Atrophic / weakly proliferative	52	1.00 (reference)	0.253		NS[*]	
Hyperplasia with atypia	20	0.62 (0.27 – 1.41)				

UPSC: uterine papillary serous carcinoma; LVSI: lymphovascular space invasion; [#]Variables entered in multivariable model; [*]Not selected because it was dropped out during stepwise multivariable analysis.

In a Cox proportional hazard model the clinicopathologic variables were analyzed for their association with progression free and overall survival. In univariable analyses, FIGO stage, mixed UPSC histology (analyzed both as continuous and categorical variable), LVSI, tumor diameter (analyzed both as continuous and categorical variable), and hyperplastic endometrium with atypia were significantly associated with both PFS and OS (Table 3). In contrast, no significant associations were found between survival and age at diagnosis, myometrial invasion, tumor in a polyp, or with

other histopathologic characteristics (hobnail cells, psammoma bodies, number of mitoses, nuclear atypia) (Table 3 and data not shown).

Table 3: Crude hazard ratios (HR) with 95% confidence interval (CI) of Progression Free Survival (PFS) and Overall Survival (OS) by clinicopathological variable of uterine papillary serous carcinoma patients, using univariable Cox regression.

		PFS		OS	
Variable	***N***	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**
Age at diagnosis	108	1.00 (0.97 – 1.03)	0.834	1.00 (0.98 - 1.03)	0.812
FIGO stage					
I – II	56	1.00 (reference)	< 0.001	1.00 (reference)	< 0.001
III - IV	52	**5.02 (2.76 – 9.13)**		**5.04 (2.78 – 9.15)**	
Histology					
Pure UPSC	50	1.00 (reference)	< 0.001	1.00 (reference)	0.001
Mixed UPSC	58	**0.37 (0.21 – 0.64)**		**0.37 (0.21 – 0.66)**	
LVSI					
No	55	1.00 (reference)	0.001	1.00 (reference)	< 0.001
Yes	53	**2.64 (1.52 – 4.61)**		**2.92 (1.68 – 5.08)**	
Myometrial invasion					
≤1/2 myometrial	57	1.00 (reference)	0.117	1.00 (reference)	0.141
>1/2 myometrial	51	1.54 (0.90 – 2.63)		1.50 (0.88 – 2.55)	
Tumor diameter	93	**1.24 (1.08 – 1.42)**	0.002	**1.28 (1.13 – 1.45)**	< 0.001
Uninvolved endometrium					
Atrophic / weakly proliferative	71	1.00 (reference)	0.023	1.00 (reference)	0.024
Hyperplasia with atypia	21	**0.34 (0.13 – 0.86)**		**0.34 (0.13 – 0.87)**	

UPSC: uterine papillary serous carcinoma; LVSI: lymphovascular space invasion.

On multivariable survival analysis, both FIGO stage (HR 4.81, 95%CI 2.34 – 9.89) and mixed UPSC histology (HR 0.35, 95%CI 0.18 – 0.67) were significantly and independently associated with PFS, whereas FIGO stage (HR 4.67, 95%CI 2.25 – 9.70), mixed UPSC histology (HR 0.39, 95%CI 0.20 – 0.76), and tumor diameter (both as continuous and categorical variable) (HR 1.21, 95%CI 1.02 – 1.43) were independently associated with OS (Table 4). Adjusting for covariates, patients with pure UPSC had a 2.6 times greater risk of death compared with mixed UPSC patients.

Finally, survival outcomes of mixed versus pure stage I-IV UPSC patients were estimated using the Kaplan-Meier method. Figure 1A demonstrates Progression Free Survival (PFS) and Figure 1B Overall Survival (OS), divided by sub-group. The PFS for mixed UPSC patients was 24 months, compared to 9 months for pure UPSC patients ($p < 0.001$, Log-rank test). In addition, the OS was significantly shorter in the group of pure UPSC patients (18 months) compared to mixed UPSC patients (74 months) ($p < 0.001$, Log-rank test). Furthermore, we also performed sub-analyses in

relation to survival using 100% UPSC histology ($N = 50$), compared to 10-40% UPSC histology ($N = 15$), and 50-90% UPSC histology ($N = 43$). Again, 10-40% UPSC and 50-90% UPSC had a favorable outcome compared to 100% UPSC for both PFS (28, 23, and 9 months, respectively) ($p = 0.001$, Log-rank test) and OS (78, 67, and 18 months, respectively) ($p = 0.002$, Log-rank test).

Table 4: Adjusted hazard ratios (HR) with 95% confidence interval (CI) of Progression Free Survival (PFS) and Overall Survival (OS) by clinicopathological variable of uterine papillary serous carcinoma patients, using multivariable Cox regression with selection procedure.

		PFS[#]		OS[#]	
Variable	***N***	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**
FIGO stage					
I - II	41	1.00 (reference)	< 0.001	1.00 (reference)	< 0.001
III - IV	40	**4.81 (2.34 – 9.89)**		**4.67 (2.25 – 9.70)**	
Histology					
Pure UPSC	36	1.00 (reference)	0.002	1.00 (reference)	0.006
Mixed UPSC	45	**0.35 (0.18 – 0.67)**		**0.39 (0.20 – 0.76)**	
Tumor diameter	81	NS[*]		**1.21 (1.02 – 1.43)**	0.028

UPSC: uterine papillary serous carcinoma; [#]FIGO stage, histology, LVSI, tumor diameter, and uninvolved endometrium were entered in the multivariable model; [*]Not selected because it was dropped out during stepwise multivariable analysis.

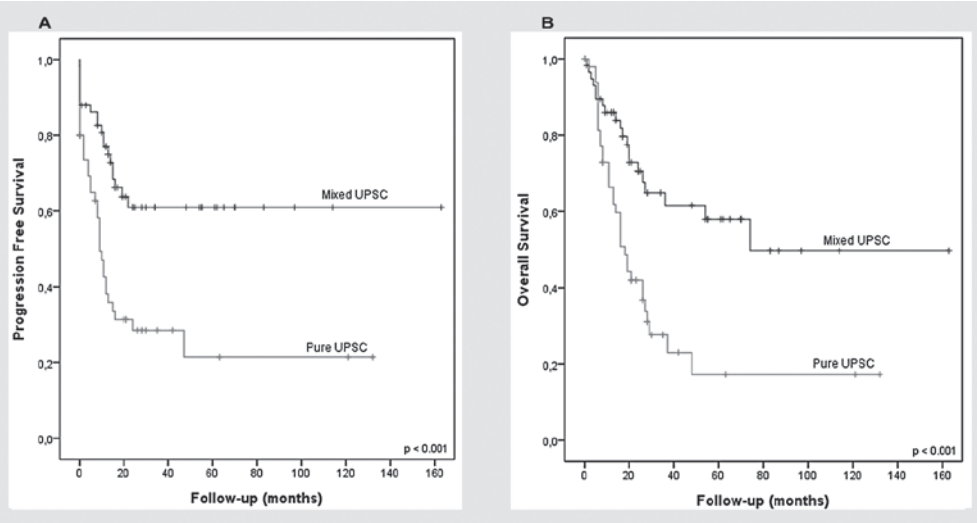

Figure 1: Kaplan-Meier estimates of **A)** progression free survival and **B)** overall survival of patients with uterine papillary serous carcinoma, showing pure uterine papillary serous carcinoma histology (100% serous component; $N = 50$, lower line) and patients with mixed UPSC histology (10–90% serous component; $N = 58$, upper line). Log-rank test $p < 0.001$ for both panels. Vertical bars indicate patients with censored data.

DISCUSSION

In the current study, we identified pure UPSC histology and FIGO stage as most important prognostic risk factors for recurrence and survival in UPSC patients. Furthermore, the precursor lesion EIC was equally found among pure and mixed UPSC cases, whereas the non-neoplastic endometrium was predominantly athrophic/weakly proliferative in pure UPSC cases compared to more hyperplastic with atypia in mixed UPSC cases.

It has been suggested that even when a minor part of the endometrial carcinoma is composed of serous histology, the patient has the same prognosis and risk for metastases as patients with pure UPSC.[6,8-11,13,14,27-29] The current study of 108 extensively reviewed stage I-IV UPSC patients shows that, besides an effect of stage of disease on recurrence risk and survival rate, mixed UPSC histology had an independent and favorable impact on the risk of recurrence and survival. There were no significant differences in demographic, clinical, or pathologic variables among mixed versus pure UPSC cases. To compare our findings with previous studies, we performed survival sub-analyses for mixed versus pure UPSC histology in both early (stage I-II) and advanced (III-IV) stage of disease. Importantly, in both early and advanced stage, mixed UPSC cases had a favorable outcome compared to pure UPSC for both PFS ($p < 0.001$, Log-rank test) and OS ($p < 0.001$, Log-rank test) (data not shown). In addition, we performed a sub-analysis among mixed UPSC cases whether the other component had an impact on survival outcome. There was a trend for a favorable outcome in mixed endometrioid-UPSC cases compared to mixed clear-cell-UPSC and mixed undifferentiated-UPSC cases, although not significant (data not shown).

Histopathologic studies with limited number of UPSC patients have suggested that the majority of serous carcinoma develop from a distinctive precursor lesion termed EIC.[21-23] We confirm this finding in our cohort of UPSC patients with EIC clearly identifiable and present in 83.9% of the cases. However, the role of EIC in the development of mixed UPSC has been largely unclear, and it could be expected that the etiology and pathogenesis of mixed UPSC may differ from that of pure UPSC. Interestingly, in the present study we show the incidence of EIC was similar in both pure and mixed UPSC cases. Endometrioid endometrial carcinoma is known to arise in a background of hyperplastic endometrium. In the present study, the endometrium adjacent to the carcinoma was mostly atrophic/weakly proliferative in cases with pure UPSC histology, in contrast to more hyperplastic endometrium with atypia in mixed UPSC cases, especially in mixed endometrioid-UPSC cases. This is in concordance with previous smaller studies.[22,27] Others suggested that mixed endometrioid-UPSC may begin as endometrioid carcinomas that arise from atypical hyperplasia, and that serous differentiation develops secondarily through a process of dedifferentiation and clonal evolution.[23] However, the hyperplastic endometrium with atypia and the serous carcinoma in both pure and mixed UPSC cases were usually topographically unrelated and appeared distinct. Together with the high incidence of EIC also in mixed UPSC cases, most often multifocally present and in direct continuity with the serous tumor, we suggest these findings do not support the etiology of mixed

UPSC arising from atypical hyperplastic endometrium via an endometrioid carcinoma within the uterine corpus.

Several limitations of our study have to be acknowledged. First, this was a retrospective cohort analysis. Not all UPSC patients were radically debulked and comprehensively staged for reasons like massive spread of disease, morbid obesity or medical co-morbidities. However, we found no difference in the percentage fully staged patients among mixed versus pure UPSC patients. In addition, similar results for clinicopathological variables were found when analyzing only completely staged pure versus mixed UPSC cases. In this study UPSC patients were included over a long period of time (i.e. 18 years), during which treatment modalities have changed, facts that could have impacted on risk for relapse and survival. We divided the UPSC population into 2 groups: group 1 included patients from the years 1992-2000 ($N = 49$) and group 2 included patients from the years 2001-2009 ($N = 59$). We found no significant differences in recurrence rate or survival (data not shown). A significant difference in follow-up period was found between pure versus mixed UPSC cases. This difference can be explained by the more aggressive nature of pure UPSC, with its higher mortality rate, and worse survival rate compared to mixed UPSC patients. The strengths of this multicentre observational study are that it comprises patients of five different institutions, with histopathology confirmed by four dedicated expert gyneco-pathologists. In the literature to date, most studies on UPSC were conducted using histopathology reports on initial diagnosis without review. To put this into perspective, within our study the number of mixed UPSC cases (10-90% serous component) increased from 38.1%, based on the initial histopathology report, to 53.7% after extensive review.

In conclusion, pure UPSC histology, together with stage of disease, are the most important prognosticators for recurrence risk and survival in serous carcinoma of the uterine corpus. Although our data show differences in recurrence risk and survival in pure compared to mixed UPSC, we suggest any endometrial carcinoma with serous differentiation must still be regarded as having high metastatic potential and should be surgically staged. Furthermore, EIC as precursor was found in both pure and mixed UPSC cases, suggestive for its role in the carcinogenesis of both types of serous endometrial carcinoma. Further histopathologic and molecular studies are needed to address whether or not pure and mixed UPSC are true different entities, with their own etiology and pathogenesis.

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Chapter 6

Tubal epithelial lesions in salpingo-oophorectomy specimens of BRCA-mutation carriers and controls

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ABSTRACT

Objective

A precursor lesion for ovarian carcinoma, serous tubal intraepithelial carcinoma (STIC), has been identified in BRCA-mutation carriers undergoing prophylactic bilateral salpingo-oophorectomy (pBSO). Other lesions were also identified in fallopian tubes, but different terminology, interpretation, and lack of knowledge of normal epithelium, have hampered to unravel their possible role in carcinogenesis. The aim of this study is to classify tubal epithelial lesions in BRCA-mutation carriers and controls to enable comparison of prevalence, area of localization, and possible malignant potential.

Methods

Two hundred twenty-six BRCA1/2-mutation carriers were included; ovaries and fallopian tubes, embedded completely, were reviewed. Controls included 105 women who underwent BSO for non-malignant reasons. Tubal epithelial lesions included the following categories: hyperplasia, minor epithelial atypia, STIC, and invasive carcinoma.

Results

Tubal neoplasia was identified in 7.1% (invasive carcinoma, 0.9%; STIC, 6.2%) of BRCA-mutation carriers compared to none in controls ($p = 0.004$). Hyperplasia and minor epithelial atypia were identified in 41.6% BRCA-mutation carriers and compared to 58.1% in controls ($p = 0.005$). Invasive carcinoma and STIC showed preference for the fimbrial ends ($p = 0.027$), while hyperplasia and minor epithelial atypia displayed more variation in localization.

Conclusions

Invasive tubal carcinoma and STIC were limited to BRCA-mutation carriers, whereas hyperplasia and minor epithelial atypia were commonly found in both BRCA-mutation carriers and controls. It is suggested that hyperplasia and minor atypia represent variations of normal tubal epithelium instead of premalignant lesions. Furthermore, total salpingectomy is strongly recommended as most but not all STIC occurred in the fimbriae.

INTRODUCTION

Ovarian carcinoma is a highly aggressive disease which is most often diagnosed in an advanced stage. The majority of cases exhibit serous histology which can either be ovarian, fallopian tube, or peritoneal in origin.[1] Until now these three localizations of serous carcinomas are considered as a single disease entity ("ovarian carcinomas") with respect to treatment and prognosis. Over the last decades the pathogenesis of ovarian carcinoma has been subject to debate. The traditional view of ovarian carcinogenesis assumes that ovarian carcinoma originates from the ovarian surface epithelium (mesothelium), which invaginates into the underlying stroma, resulting in epithelial inclusion cysts that eventually undergo malignant transformation.[2] However, these epithelial inclusion cysts were invariably present in both high risk cases and controls.[3-5] Recently, a predisposition for ovarian carcinoma was discovered in the fallopian tubes of women with a germline BRCA-mutation.[6,7] Prophylactic bilateral salpingo-oophorectomy (pBSO) is performed in the majority of BRCA-mutation carriers, which reduces the risk of ovarian carcinoma by 80%.[8,9] Incidental findings of occult invasive carcinoma and precursor lesions such as serous tubal intraepithelial carcinoma (STIC) were found in prophylactically removed fallopian tubes from women with a BRCA-mutation.[6,7] Recent studies have established that STIC is present in 1-17% of these women.[10-17]

Histopathological investigation of fallopian tube specimens of women diagnosed with serous ovarian carcinomas reported the presence of STIC in up to 50% of these women.[11,18,19] Recent morphologic and molecular genetic studies provided compelling evidence that a proportion of primary serous ovarian tumors actually arise from these precursor lesions; unique and identical p53 mutations were found in both the serous ovarian carcinoma and corresponding STIC, indicating a monoclonal relation.[20-22] It is hypothesized that (pre)malignant cells of the tubal epithelium may exfoliate into the tubal lumen and migrate by retrograde flow to the abdominal cavity; implantation of these cells onto the peritoneum or ovarian surface might result in the development of serous carcinomas.[8,20,23]

Histological entities other than STIC were also described in the tubal epithelium, for which various terminologies were used such as dysplasia, atypical hyperplasia or mucosal epithelial proliferations.[15] Because of difficulties in interpretation it is still unclear whether all these subtypes have a causal role in the development of tubal or ovarian carcinoma. Furthermore, because most studies did not include a proper control group of healthy women without a BRCA-mutation, knowledge of normal tubal epithelium is poor. This knowledge of tubal epithelial lesions in the general population is essential to enable differentiation of true premalignant lesions from lesions representing variants of normal tubal epithelium.

In this study, we report on tubal epithelial lesions in the largest cohort to date of women with a confirmed BRCA 1/2 mutation who underwent pBSO, in comparison to a large control group of women that underwent BSO for non-malignant reasons. Identified prevalences and areas of localization in the tubes will be compared between both groups to enable identification of lesions representing possible premalignancies and lesions that are most likely variations of normal tubal epithelium. In addition, a scheme for classification of tubal epithelial lesions is suggested.

MATERIALS & METHODS

Clinical data

Between January 1996 and December 2009, all women with a known germline BRCA1/2 mutation opted for pBSO at the Radboud University Nijmegen Medical Centre (RUNMC), The Netherlands, were identified. Patients with surgery for preoperatively suspected ovarian or tubal lesions were not included in this study. The Dutch nation-wide pathology database (PALGA) was used to identify a control group of women who underwent BSO at the RUNMC between 1999 and 2009. The control group included specimens of 105 women who underwent hysterectomy and/or salpingo-oophorectomy with a non-malignant diagnosis: uterine leiomyomatosis (*N* = 42), mucinous cystadenoma (*N* = 19), serous cystadenoma (*N* = 14), fibroma (*N* = 6), endometrial hyperplasia with or without atypia (*N* = 6), mature teratoma (*N* = 5), ovarian torsion (*N* = 4), endometriosis (*N* = 3), uterovaginal prolapse (*N* = 3) or gynecological complaints such as meno- or metrorhagia without a distinct histopathologic diagnosis (*N* = 3).

Clinical and histopathological data were collected for each patient using a standardized form. Data collected included the BRCA-mutation status, age, menopausal status, presence of earlier breast carcinoma and management of the breast carcinoma. On behalf of the research ethics committee of the RUNMC, the study has been carried out in accordance with the applicable rules concerning the review of research ethics committees and informed consent.

Pathology

All pBSO specimens of BRCA-mutation carriers were completely embedded, according to standard protocol. The fallopian tubes were cross sectioned at 3 mm interval, except for the fimbrial ends that were sectioned longitudinally to enable maximum exposure of the tubal plicae. One haematoxylin & eosin stained section was produced from each paraffin block. Fallopian tube specimens of control cases were not completely embedded. From these fallopian tubes representative sections of the fimbria, ampulla and/or isthmus were selected and embedded for histological examination. All available tubal sections were reviewed by two pathologists (MB, JB) with ample experience in gynecological pathology, being blinded for clinical patient characteristics. Tubal or ovarian epithelial lesions and their locations were recorded. Consensus was reached concerning the criteria for tubal epithelial lesions before the start of the present study. In case of discrepant diagnoses consensus was reached between both pathologists. In the present study, the following entities were distinguished in the fallopian tubes: benign epithelium, hyperplasia, minor epithelial atypia, STIC (tubal intraepithelial carcinoma, non invasive severe dysplasia and carcinoma *in situ*), and (occult) tubal invasive carcinoma.

Benign fallopian tube

The normal fallopian tube is lined by non stratified epithelium with a mixture of three cell types (Figure 1). Ninety percent of the tubal mucosa is layered by secretory and ciliated cells. The third cell type is the intercalated cell, which is inconspicuous and considered to be a secretory cell as well.

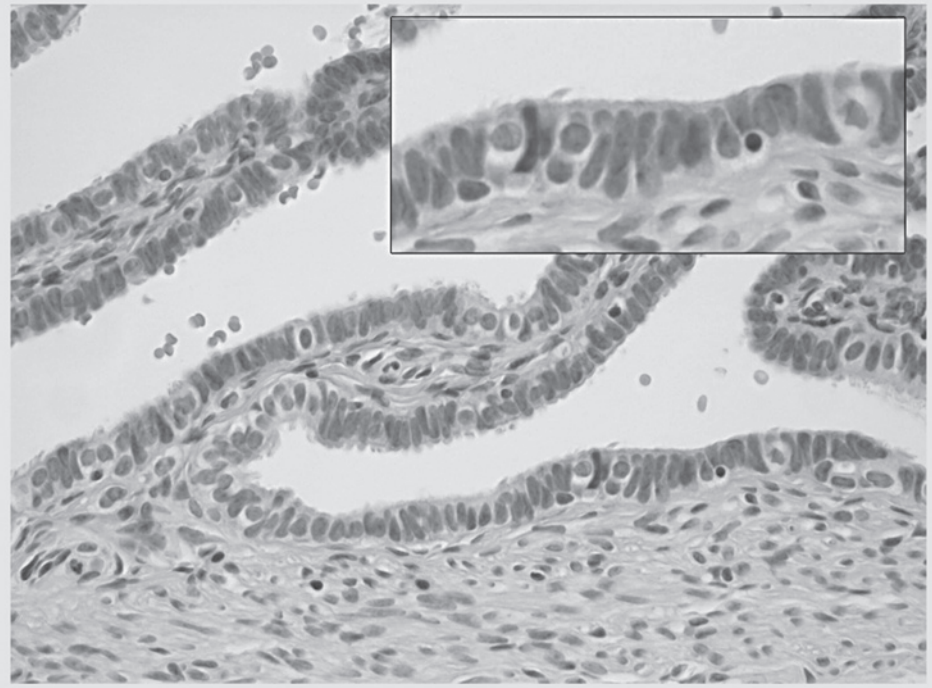

Figure 1: Benign fallopian tube epithelium with secretory and ciliated cells (level of magnification: 200×; level of magnification of inset: 400×).

Hyperplasia

Tubal hyperplasia is defined as cellular crowding, stratification, occasional tufting of cells, some loss of nuclear polarity, but the absence of nuclear atypia. Cellular crowding is visible when the number of secretory cells exceeds the number of ciliated cells (Figure 2A).

Minor epithelial atypia

Features for histological diagnosis of minor epithelial atypia are slightly enlarged, rounded nuclei with irregular cell membrane outlines, slightly enlarged nuclear/cytoplasm ratio, nuclei with slight loss of polarity and inconspicuous nucleoli (Figure 2B). Minor epithelial atypia is not visible at low power magnification. It comprises epithelial lesions that fulfill some but not all of the criteria for STIC.

Tubal intraepithelial carcinoma (STIC)

Tubal intraepithelial carcinoma is identifiable at low power magnification, displaying a row of dark and thickened epithelium. It is characterized by disorganized cellular crowding and nuclear

stratification, and consists of secretory cells in absence of ciliated cells. Other features include mitotic figures, high nuclear/cytoplasm-ratio, nuclear pleomorphism with loss of polarity and prominent nucleoli (Figure 2C).

Tubal invasive carcinoma

Histological features of tubal invasive carcinoma are identical to STIC, but with the addition of an invasive component (Figure 2D).

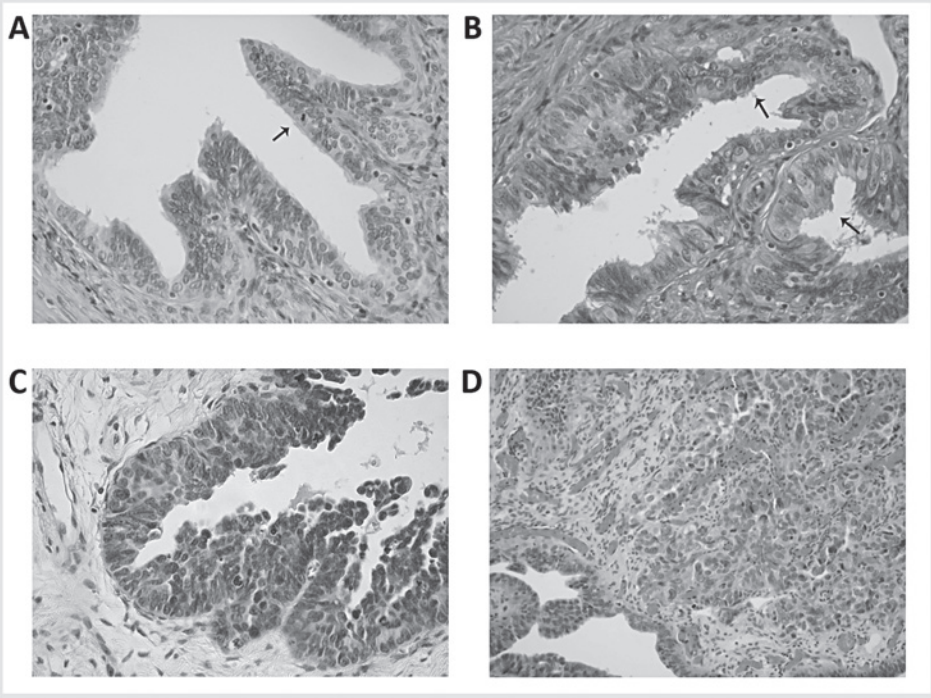

Figure 2: Tubal epithelial lesions (level of magnification: 200×). **A)** Fallopian tube epithelium with hyperplasia, showing cellular crowding, but without atypical nuclei (arrow: mitotic figure). **B)** Minor epithelial atypia in the fallopian tube showing cellular crowding, slight cellular atypia and nuclei with small nucleoli, but without loss of polarity of the epithelial cells (arrows). **C)** Tubal intraepithelial carcinoma (STIC) with cellular crowding, stratification of nuclei, loss of polarity and severe atypia of the nuclei. **D)** Fallopian tube carcinoma, including overlying atypical epithelium.

Statistical analysis

Pearson's chi square and, if appropriate, the Fisher's exact test were used to test associations between categoric variables. The Kruskal-Wallis and Mann-Whitney *U* test were used to compare medians between different independent samples. All statistical analyses were performed using SPSS software version 18.0 (SPSS Inc, Chicago, IL) and $p < 0.05$ (two-sided test) was considered statistically significant.

RESULTS

Clinical findings

A total number of 226 women with a confirmed BRCA-mutation were included in the current study (149 BRCA1 and 77 BRCA2 mutations). Also, 105 controls were included. Women with a BRCA-mutation had a median age of 44 years (range 24-70) compared to controls with a median age of 48 years (range 22-60) ($p = 0.04$, Table 1). The median age specified for type of BRCA-mutation was 42 years for the BRCA1 mutation carriers and 48 years for the BRCA2 mutation carriers. Distribution of menopausal status was comparable for both groups; 40% of the BRCA-mutation carriers were postmenopausal and 32% of the controls (85 of 212 vs 33 of 103; $p = 0.17$, Table 1). Median number of parity was two for both BRCA-mutation carriers and controls, but the BRCA-mutation carriers had more often three or more children compared to controls (Table 1). Breast carcinoma prior to BSO was significantly more often diagnosed in BRCA-mutation carriers compared to controls (81 of 226 (36%) *vs* 1 of 105 (1%); $p < 0.001$, Table 1).

Table 1: Population characteristics of BRCA-mutation carriers (*N* = 226) and controls (*N* = 105).

	BRCA 1/2	Controls	
Population characteristics	***N* (%)**	***N* (%)**	***p*-value**
Median age (range)	44 (24-70)	48 (22-60)	**0.04**[a]
Menopausal status			**0.17**[b]
Premenopausal	127 (60%)	70 (68%)	
Postmenopausal	85 (40%)	33 (32%)	
Missing	14	2	
Parity			**0.001**[b]
No children	34 (16%)	23 (24%)	
1-2 children	110 (50%)	60 (62%)	
3+ children	75 (34%)	14 (14%)	
Missing	7	8	
History of breast carcinoma	81 (36%)	1 (1%)	**<0.001**[b]
Sterilization	23 (10%)	17 (16%)	0.11[b]

[a]Mann Whitney U test; [b]Pearson chi square.

Tubal epithelial lesions

The median number of sections reviewed from the fallopian tubes was 17 (range 2-33) for BRCA-mutation carriers and six (range 1-20) for controls ($p < 0.001$). Of the 226 women with a BRCA-mutation, 116 (51%) were diagnosed with benign tubal epithelium and in 110 (49%) a tubal epithelial lesion was detected: 43 (19%) cases showed hyperplasia, 51 (23%) minor epithelial atypia, 14 (6%) a STIC, and two (1%) an invasive tubal carcinoma (Table 2). In seven cases STIC was identified bilaterally

and in another seven cases unilaterally. Of the seven cases with unilateral STIC, the contralateral tubal epithelium showed benign epithelium in three cases, hyperplasia in one, and minor epithelial atypia in three. Two cases were identified with invasive tubal carcinoma which occurred unilaterally with contralaterally benign tubal epithelium in one case and occult ovarian carcinoma in the other. In the control group the tubal epithelium was benign in 44 (42%) cases, showed hyperplasia in 37 (35%) and minor epithelial atypia in 24 (23%) cases. No tubal neoplasia (STIC or invasive carcinoma) was found in controls, which was significantly different from the BRCA-mutation carriers (zero of 105 *vs* 16 of 226 (7%); $p = 0.004$). The less severe lesions, hyperplasia and minor epithelial atypia, were commonly identified in both BRCA-mutation carriers and controls, although more often in controls (94 of 226 (42%) *vs* 61 of 105 (58%); $p = 0.005$, Table 2).

Table 2: Histopathologic findings in the epithelium of the fallopian tubes from both BRCA-mutation carriers and controls that received bilateral salpingo-oophorectomy.

	BRCA1 (*N* = 149)		BRCA2 (*N* = 77)		BRCA1/2 (*N* = 226)		Controls (*N* = 105)	
Tubal Epithelium	***N***	**(%)**	***N***	**(%)**	***N***	**(%)**	***N***	**(%)**
Benign	80	(53.7%)	36	(46.8%)	116	(51.3%)	44	(41.9%)
Hyperplasia	29	(19.5%)	14	(18.2%)	43	(19.0%)	37	(35.2%)
Minor atypia	31	(20.8%)	20	(26.0%)	51	(22.6%)	24	(22.9%)
STIC	9	(6.0%)	5	(6.5%)	14	(6.2%)	0	(0.0%)
Invasive carcinoma	0	(0.0%)	2	(2.6%)	2	(0.9%)	0	(0.0%)

STIC: serous tubal intraepithelial carcinoma

Furthermore, severity of tubal epithelial lesions in women with a BRCA-mutation was significantly associated with higher age ($p < 0.001$, Table 3). Controls revealed no significant difference in age distribution for tubal epithelial lesions (Table 3).

Table 3: Tubal epithelial lesions in BRCA-mutation carriers and controls in relation to age.

	BRCA 1/2		Controls	
Tubal Epithelium	***N***	**Median age (range)**	***N***	**Median age (range)**
Benign	116	41.5 (24-70)	44	48.0 (28-59)
Hyperplasia	43	45.0 (36-70)	37	48.0 (35-60)
Minor atypia	51	48.0 (35-69)	24	51.0 (22-59)
STIC	14	53.0 (35-63)	-	
Invasive carcinoma	2	57.5 (55-60)	-	
		$\mathbf{p < 0.001}^{\$}$		$p = 0.14^{\$}$

STIC: serous tubal intraepithelial carcinoma

Topographic characteristics of tubal epithelial lesions

In the majority of cases, invasive tubal carcinoma and STIC were identified in the distal fimbria: invasive tubal carcinomas in 100% and STICs in 64% of the cases (Table 4). Tubal neoplasia (invasive carcinoma or STIC) was significantly more often localized in the fimbriae compared to hyperplasia and minor epithelial atypia (11 of 16 (69%) *vs* 62 of 155 (40%); $p = 0.027$, Table 4). As mentioned previously, invasive tubal carcinoma and STIC were only identified in BRCA-mutation carriers and not in the controls. In the BRCA-mutation carriers hyperplasia was seen in the fimbriae in 35% and minor epithelial atypia in 47%, versus 27% and 54% in the controls, respectively. Hyperplasia and minor epithelial atypia did not occur significantly more often in the fimbriae but displayed more variation in localization (39 of 94 (41%) *vs* 23 of 61 (38%); $p = 0.64$, Table 4).

Ovarian occult carcinoma

An occult ovarian carcinoma of 5.8 mm was identified in a 60 year old woman with a BRCA2 mutation, with an occult invasive carcinoma in one of the adjacent tubes. All other cases, from both the BRCA-mutation and control group, showed only benign ovarian pathology.

Of BRCA-mutation carriers, 81 (36%) were diagnosed with breast carcinoma prior to pBSO. In these 81 BRCA-mutation carriers STIC was present in 5% and invasive tubal carcinoma in 2%, compared to respectively 7% and 0% in the 133 BRCA-mutation carriers without prior breast carcinoma. The presence of tubal neoplasia (STIC and invasive carcinoma) was not significantly different for cases with or without a previous breast carcinoma (six of 81 (7%) *vs* nine of 133 (7%); $p = 0.86$). Treatment for breast carcinoma was surgery (36%), or in addition either adjuvant chemotherapy (49%) or adjuvant chemotherapy with tamoxifen (15%). No significant difference was seen between treatment management of breast carcinoma and prevalence of tubal neoplasia (tamoxifen, $p = 0.58$ (0/12 *vs* 6/69); chemotherapy, $p = 0.18$ (2/52 *vs* 4/29)).

Table 4: Tubal epithelial lesions in BRCA-mutation carriers located in the fimbria or in the isthmus and ampullary region of the tube (non-fimbrial).

	BRCA 1/2				Controls			
	Fimbrial (*N* = 50)		Non-fimbrial (*N* = 59)		Fimbrial (*N* = 23)		Non-fimbrial (*N* = 38)	
Tubal Epithelium	***N***	**(%)**	***N***	**(%)**	***N***	**(%)**	***N***	**(%)**
Hyperplasia	15	(34.9%)	28	(65.1%)	10	(27.0%)	27	(73.0%)
Minor atypia	24	(47.1%)	27	(52.9%)	13	(54.2%)	11	(45.8%)
STIC	9	(64.3%)	5	(35.7%)	-	-	-	-
Invasive carcinoma	2	(100.0%)	0	(0.0%)	-	-	-	-

STIC: serous tubal intraepithelial carcinoma

DISCUSSION

Our study has identified significant differences in the prevalence and preferred localization of tubal epithelial lesions in a large cohort of 226 BRCA-mutation carriers versus 105 controls. Occult invasive tubal carcinoma and STIC were present in 7.1% of the BRCA-mutation carriers but were absent in control cases. In contrast, tubal hyperplasia and minor epithelial atypia were commonly identified in both BRCA-mutation carriers and controls, although more often in controls. An association was found between increasing age and the presence of more severe tubal epithelial lesions in BRCA-mutation carriers. Furthermore, invasive tubal carcinomas and STICs had a marked preference for the distal fimbrial end, which was not seen for tubal hyperplasia and minor epithelial atypia.

Our divergent results for the different categories of tubal epithelial lesions lead to the suggestion that hyperplasia and minor epithelial atypia do not represent precursor lesions of invasive carcinomas and/or STICs. Previously, it has been suggested that a stepwise epithelial carcinoma progression model can be applied to the tubes.[11] According to this model, occult carcinoma and STIC are preceded by atypic and/or hyperplastic tubal lesions. However, according to the results of the present study the accurateness of this stepwise progression model seems controversial, as prevalence of the specific tubal lesions as well as areas of localization are different for BRCA-mutation carriers and controls. The association found between increasing age and severity of tubal epithelial lesions in BRCA-mutation carriers might be suggestive for the stepwise progression model, but as the less severe lesions are more often identified in controls it seems unlikely that they are part of the oncogenic pathway. It is more likely that the less severe tubal lesions, as hyperplasia, represent normal proliferation of the tubal epithelium. Tubal hyperplasia is more common in younger women, as was suggested by Norquist *et al*.[24] The fact that invasive carcinomas and STICs were not identified in controls marks their uniqueness for women at high risk for ovarian carcinoma and therefore their suggested role in carcinogenesis, especially, since controls were older and therefore more STICs and carcinomas would be expected. In addition, histological findings of hyperplasia and minor atypia were not identified in close proximity with occult invasive carcinoma and STIC.[25]

The incidence of invasive tubal carcinoma and STIC in BRCA-mutation carriers in the present study was comparable with other studies including relatively large populations, with a mean overall prevalence of 5.2% for STIC and 6.7% for tubal neoplasia (STIC and invasive carcinoma) (Table 5). Comparing the prevalence of hyperplasia and minor epithelial atypia with previous reports entails more obstacles. Various terminologies have been used by different authors, or only distinct lesions such as occult carcinoma and STIC were recorded. Some extent of tubal epithelial proliferation has been seen in women, mostly premenopausal, without an increased risk of ovarian or tubal carcinoma, and are considered within normal limits of epithelial variation.[26] However, the extent of this normal variation is still somewhat unclear as most studies did not include a control group. The current study provided the first large control group consisting of patients that were operated for various non-malignant reasons. Only two previous studies on epithelial lesions of the fallopian tubes included a control group (Table 5).[25,27]

The study of Shaw *et al.* included 64 controls who underwent BSO and reported STIC in 3% of their control cases.[27] However, 22% of their controls underwent surgery for synchronously diagnosed carcinoma of the cervix or endometrium which could have biased their results.[27] In our own control group all patients receiving BSO for malignancy were excluded to rule out possible intrusion.

A limitation of the current study is its retrospective nature leading to less extensive sampling of tubal tissue of controls. Therefore, median number of tubal slides available for review in BRCA-mutation carriers versus controls differed, resulting in an inability of completely blinding the pathologist for BRCA status. Tubal epithelial lesions are quite small and the use of a pathological protocol to embed the fallopian tubes in toto is preferred in further prospective research in order not to overlook putative precursor lesions. One of the strengths of our study is that it included the largest cohort of both BRCA-mutation carriers and controls. Age distribution of the cohort was comparable with other studies[12,17,27], both groups were identified in one single institution and all sections were reviewed by the same two gyneco-pathologists. Before starting this study, a classification scheme for tubal epithelial lesions was developed and consensus was reached on definitions of these epithelial tubal lesions.

Although various epithelial tubal lesions in women at high risk for ovarian carcinoma were described previously, the diagnosis of tubal epithelial lesions is still subject to many difficulties as outlined in a recent report by Visvanathan *et al.*[28] They analyzed inter- and intraobserver reproducibility between gynecologic pathologist in diagnosing tubal epithelial lesions which showed to be fair to moderate for STIC, but poor for other tubal epithelial lesions.[28] To bypass definition issues several reports combined terms as occult carcinoma and STIC in their reported incidence number, which further complicates comparison of studies.[8,13,27] The difficulties in interpretation of results stresses the importance of a generally used morphologic classification for future research. Our proposed classification scheme could overcome these interpretation difficulties when generally used.

The BRCA-mutation carriers also have an increased risk of developing breast carcinoma which often occurs at an earlier age than ovarian carcinoma.[29] Literature shows some controversy on the influence of prior breast carcinoma and the risk of identifying occult tubal carcinoma in pBSO tissue.[8,16] In the current study prior breast carcinoma did not influence the presence of tubal epithelial lesions, nor did different treatment protocols for breast carcinoma.

It is recommended to perform a pBSO in women with a confirmed BRCA-mutation at the age of about 40 years, which is based on the increased prevalence of tubal or ovarian carcinoma in these women.[30] This is supported by the present study as both identified invasive tubal carcinomas occurred at the age of 55 and 60, respectively. However, in the current study, the precursor lesion STIC was identified in two women before reaching the age of 40 years. The median age of STIC was 53 years (range 35-63) and age distribution of identified STIC was shown in Table 6. Whether the identification of STIC before reaching the age of 40 years is a reason to perform pBSO at a younger age needs further investigation.

Table 5: Studies that reported on occult tubal neoplasia (invasive tubal carcinoma and STIC) in BRCA-mutation carriers and controls (with an assumed risk for ovarian or tubal carcinoma comparable to the general population).

		BRCA 1/2 mutation carriers				Controls			
Study	**Year**	***N***	**Occult neoplasia *N* (%)**	**Invasive carcinoma *N* (%)**	**STIC / CIS *N* (%)**	***N***	**Occult neoplasia *N* (%)**	**Invasive carcinoma *N* (%)**	**STIC / CIS *N* (%)**
Present study	2012	226	16 (7.1%)	2 (0.9%)	14 (6.2%)	105	0	0	0
Shaw *et al.*[27]	2009	176	15 (8.0%)	*Excl.	15 (8.0%)	64	2 (3.0%)	Excl.*	2 (3.0%)
Carcangiu *et al.*[25]	2003	26	2 (7.7%)	0 (0.0%)	2 (7.7%)	49	0	0	0
Finch *et al.*[12]	2006	159	6 (3.8%)	5 (3.1%)	1 (0.6%)	-	-	-	-
Callahan *et al.*[17]	2007	122	7 (5.7%)	7 (5.7%)	6 (4.9%)	-	-	-	-
Manchanda *et al.*[14]	2011	117	7 (6.0%)	1 (0.9%)	6 (5.1%)	-	-	-	-
Powell *et al.*[16]	2011	111	9 (8.1%)	4 (3.6%)	6 (5.4%)	-	-	-	-
Rabban *et al.*[33]	2009	102	8 (7.8%)	1 (1.0%)	7 (6.8%)	-	-	-	-
Powell *et al.*[34]	2005	67	4 (6.0%)	3 (4.5%)	3 (4.5%)	-	-	-	-
Olivier *et al.*[35]	2004	65	3 (4.6%)	3 (4.6%)	-	-	-	-	-
Lamb *et al.*[13]	2005	62	5 (8.1%)	1 (1.6%)	4 (6.5%)	-	-	-	-
Overall		1233	82/1233 (6.7%)	27/1057 (2.6%)	64/1233 (5.2%)				

STIC: serous tubal intraepithelial carcinoma; CIS: carcinoma in situ

More research is needed on the prevalence of STIC before the age of 40, on the possibility of STIC metastasizing before developing towards carcinoma and, if so, if this is an indication for adjuvant treatment in these women. Recently, 'radical fimbriectomy' was suggested as an alternative solution for prevention of tubal or ovarian carcinoma instead of pBSO.[31] However, our study contradicts that only fimbriectomy would be sufficient in preventing these malignancies. We identified only two-thirds of STICs in the fimbrial end while one-third occurred in the ampulla or isthmus of the tubes. Removal of the total fallopian tubes after completion of childbearing with a second step removal of the ovaries at a higher age might be a more suitable alternative for pBSO that needs further investigation.[32] In addition, this method could be more safely performed before the age of 40 years as recommended for pBSO, without the adverse effects of early menopause.

Table 6: Age distribution of STIC in women with a confirmed BRCA-mutation.

	BRCA1 (*N* = 149)	**BRCA2 (*N* = 77)**	**BRCA 1/2 (*N* = 226)**
Age category	***N* (total *N*)**	***N* (total *N*)**	***N* (total *N*)**
<35	0 (9)	0 (1)	0 (10)
35-40	2 (57)	0 (17)	2 (74)
41-50	2 (42)	2 (26)	4 (68)
50+	5 (41)	3 (33)	8 (74)

In conclusion, the results of the present study emphasize the essence of removing the fallopian tubes in total, as one-third of the identified STICs did not occur in the fimbrial end. Furthermore, the classification scheme used in this study appears to be useful as it differentiates between equivocal tubal epithelial lesions (hyperplasia and minor epithelial atypia) that are commonly present in the general population, from premalignant lesions with an already established role in carcinoma development (STIC and invasive carcinoma). Occult invasive carcinoma and STIC were only identified in BRCA-mutation carriers, whereas hyperplasia and minor epithelial atypia were more often identified in controls. Therefore, it seems unlikely that hyperplasia and minor epithelial atypia are also precursor lesions of ovarian carcinoma. We suggest to interpret tubal hyperplasia and minor epithelial atypia as variants of normal tubal epithelial proliferation.

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Chapter 7

Concurrent endometrial intraepithelial carcinoma (EIC) and serous ovarian cancer: can EIC be seen as the precursor lesion?

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ABSTRACT

Objective

The pathogenesis of serous ovarian carcinoma (SOC) is still unknown. Recently, endometrial intraepithelial carcinoma (EIC) was proposed to be the precursor lesion of SOC. This study examines the model of EIC as precursor for SOC.

Methods

Cases of SOC with a noninvasive or superficially invasive serous lesion, a hyperplastic lesion with/without atypia, or EIC in the endometrium were selected for inclusion in this study. Tissue sections from both ovaries, the fallopian tubes, and the uterus were extensively reviewed by an expert gynecopathologist. For both EIC and SOC, immunostaining for p53, Ki-67, estrogen receptor, and progesterone receptor; *TP53* mutation analysis; and *in situ* ploidy analysis were performed.

Results

Nine cases of SOC with concurrent EIC in the endometrium were identified. Immunostaining for p53, Ki-67, estrogen receptor, and progesterone receptor revealed almost identical expression patterns and similar intensities in each pair of EIC and coincident SOC. Identical *TP53* mutations were found in SOC and coinciding EIC in 33% of the cases, suggesting a clonal origin. DNA ploidy analysis, as a marker for neoplastic progression, demonstrated an increased number of aneuploid nuclei in SOC compared to their corresponding EIC (p = 0.039). In addition, the mean amount of DNA per nucleus in SOC was higher (i.e. more aneuploid) compared to EIC (p = 0.039).

Conclusion

This study provides a first indication of EIC as possible precursor lesion for SOC. This finding could have major clinical implications for future ovarian cancer management and underscores EIC as a possible target for early SOC detection and prevention.

INTRODUCTION

Although ovarian cancer only comprises approximately 3% of all female malignancies, it accounts for a disproportionately high mortality number, making it the leading cause of death from gynecologic cancers. Serous ovarian carcinoma (SOC) comprises approximately 70% of all ovarian cancers. Its aggressive characteristics are caused by its propensity for serosal organ involvement, discovery at an advanced stage, and peritoneal spread.

There has been an ongoing debate about the pathogenesis of SOC. Many studies have focused on the ovarian surface epithelium, mainly supported by epidemiologic findings: a reduction in the number of ovulations as a result of multiple pregnancies, breast feeding, and the use of oral contraceptives lead to a decreased risk for developing ovarian cancer.[1,2] Constant ovulation-induced damage and repair of the ovarian surface was suggested as the source of malignant transformation of ovarian epithelium.[3,4] However, experimental or histopathological evidence is lacking, and a precursor lesion was never identified in the ovary itself. Recently, evidence has accumulated supporting the model of origin for a subset of high-grade ovarian serous carcinoma in the distal fallopian tube.[5,6] Several studies identified an early form of serous carcinoma in the fimbriated end of the fallopian tube of female BRCA-mutation carriers, and this precursor lesion was termed serous tubal intraepithelial carcinoma (STIC).[7-9]

We recently described an alternative hypothesis regarding the site of origin for SOC in which endometrial intraepithelial carcinoma (EIC) was proposed to be the precursor lesion.[10] Endometrial intraepithelial carcinoma was originally designated as the precursor lesion of uterine papillary serous carcinoma (UPSC) and was found near or adjacent to UPSC in 50% to 90% of the cases.[11,12] Endometrial intraepithelial carcinoma is noninvasive, often multifocal in origin, and can also be found on the surface of the ovary and in the fallopian tube.[11,13] It was presumed that coexistence of multifocal serous carcinoma of the ovaries and endometrium originated from a single rather than from multiple sites.[14] Therefore, we postulate that transtubal migration of the loosely cohesive cells from EIC foci in the endometrium may be the basis for the development of any type of intraperitoneal serous carcinoma. This is strengthened by the observations that hysterectomy without oophorectomy and tubal ligation both have been associated with reductions in the risk for ovarian cancer; odds ratios have ranged from 0.03 to 0.8 for hysterectomy and from 0.2 to 0.9 for tubal ligation.[15-20] Mutation and altered expression of the tumor suppressor protein p53 may be involved in the development of EIC and SOC. *TP53* gene mutation analysis revealed identical mutations in both EIC and the concordant serous pelvic or ovarian carcinoma in a small cohort of patients, supporting a clonal origin.[21] In a different study, identical *TP53* mutations were shown in tumor foci from patients with peritoneal dissemination and ovarian involvement in association with a noninvasive serous endometrial lesion.[14] The present study aims to further clarify the possible relationship between SOC and the presence of concurrent EIC in the endometrium by performing protein expression analysis using immunohistochemistry, *TP53* mutation analysis, and *in situ* DNA ploidy analysis.

MATERIALS & METHODS

Case selection

Patients with unilateral or bilateral SOC, diagnosed and surgically treated at the Radboud University Nijmegen Medical Center during the period 1993 and 2009 were selected (*N* = 192). Without revision of the primary histology at this stage, cases were selected with (1) a noninvasive or superficially invasive serous lesion, a hyperplastic lesion with/without atypia, or EIC in the endometrium described in the original pathology report, and (2) tissue from both ovaries, the fallopian tubes in toto, and the uterus were available for revision. Thirty-eight cases fulfilled these criteria, and hematoxylin-and-eosin-stained sections of all tissues were extensively reviewed for the presence of EIC by an expert gynecopathologist (JB), using previously published criteria.[11,22] A total of 9 cases with SOC and concurrent EIC were identified and included in this study. For each case, patients' demographics and clinicopathological findings were determined from our institution's medical and operative records, including age, International Federation of Gynecology and Obstetrics (FIGO) stage, and the extent of the ovarian carcinoma, lymphovascular space invasion (LVSI), and the number of endometrial slides reviewed.

Immunohistochemistry

For assessment of protein expression by immunohistochemistry, 4-µm tissue sections were prepared from archival, formalin-fixed, paraffin-embedded tissue of both SOC and EIC and processed using standard techniques. A p53 monoclonal antibody (clone DO-7, Neomarkers, Fremont, CA) was used at a dilution of 1:250. A monoclonal antibody for Ki-67 (clone MIB-1, Dako, Glostrup, Denmark) was used at a dilution of 1:200; and for estrogen receptor (ER; clone SP-1, Thermo Scientific/Lab Vision, Fremont, CA), a monoclonal antibody was used at a dilution of 1:100. A monoclonal antibody for progesterone receptor (PR; clone PgR636, Dako) was used at a dilution of 1:500. After the application of appropriate secondary antibodies, a standard streptavidin-biotin technique (Vector Laboratories, Burlingame, CA) with diaminobenzidine as chromogen was used. All slides were counterstained with hematoxylin. Protein expression was scored as both the percentage of positively stained cells and the intensity of staining (-, negative; +, slight; ++, moderate; and +++, strong). For Ki-67, the percentage of immunostaining was assessed by average of 5 high-power fields. All slides were analyzed by 2 independent observers (TR and LvK).

Analysis of p53 mutation status

Both the SOC and its concordant EIC were analyzed for *TP53* mutations. In all cases, normal endometrium/myometrium and normal ovarian stroma served as reference tissue to allow the identification of possible somatic mutations. Tissue sections (7 µm) were mounted on glass-foiled polyethylene naphthalate membrane-covered glass slides (Leica, Rijswijk, The Netherlands) and stained with cresyl violet (Applied Biosystems/Ambion, Austin, TX) according to suppliers instructions. After drying, sections were covered with PinPoint solution (ZymoResearch, Orange, CA).

Laser-capture microdissection (LMD6000, Leica Microsystems, Wetzlar, Germany) was performed to isolate clusters of cells for genetic analysis. DNA isolation was performed as previously described.[23] Genomic DNA was amplified by polymerase chain reaction using M13-tailed primers designed to amplify exons 5 to 8 of *TP53*.[23] Polymerase chain reaction products were sequenced from both strands using M13 primers. Data were analyzed using Chromas software (Version 1.45, Griffith University, Queensland, Australia). Candidate mutations found by the software were compared with a reference database for cancer-associated *TP53* mutations (International Agency for Research on Cancer *TP53* Database, http://www-p53.iarc.fr/). Samples were scored as *TP53* mutation-positive only if an identical mutation was identified in both the forward and reverse sequences.

DNA ploidy assessment in tissue sections

Measurement of the DNA ploidy status was performed as described previously.[24,25] In brief, 7-µm thick paraffin-embedded tissue sections were incubated with primary antibody directed against Ki-67 (clone MIB-1, Dako). Subsequently, nuclear DNA was stoichiometrically stained using DRAQ-5 (Biostatus Limited, Leicestershire, UK). For each case, representative areas were selected by an experienced gynecopathologist (JB) and consisted of the following: (1) areas classified histopathologically as EIC with (2) areas containing normal endometrium/myometrium as diploid reference tissue, and (3) areas containing SOC with (4) areas containing normal ovarian stroma as diploid reference tissue. Images of DRAQ-5 and Ki-67 were acquired simultaneously using fluorescence microscopy. Automated recognition of nuclei was performed, and if required, results of automatic segmentation were interactively corrected. For nuclei in EIC and SOC, DNA index (DI) values were calculated using the respective diploid reference tissues (DI = 1.00 means diploidy). To be able to differentiate between proliferating euploid cells and aneuploid cells, only Ki-67-negative nuclei were analyzed. Those nuclei in EIC and SOC with DI greater than 1.25 were considered aneuploid. From these, the percentage of aneuploid cells (extent of aneuploidy) and the average DI value (degree of aneuploidy) were calculated.

Statistics

All data analyses were performed using SPSS software (version 16.0, SPSS Inc, Chicago, IL). The Fisher exact test was used to calculate *p*-values for association of p53 expression between EIC and SOC. To compare the extent and degree of aneuploidy between EIC and SOC, the Sign test was used.

RESULTS

Clinical findings

Nine cases of previously diagnosed SOC fulfilling our criteria were identified. In each case, noninvasive or microinvasive EIC was confirmed in the uterine specimen, whereas none of the cases exhibited a STIC in their fallopian tubes after examination. In 2 of the 9 cases, EICs were partially microinvasive.

However, based on the total extensiveness of the EIC in the various histopathological slides in these 2 cases, and after review by our expert gyneco-pathologist, the lesions in the endometrium did not meet the criteria for minimally invasive UPSC. Therefore, we denoted these lesions as microinvasive EIC. Table 1 summarizes the results of histopathological examination of the endometrium and fallopian tubes of the included patients. A median of 5 endometrial sections (range 2-10) of each uterus were evaluated. The mean age of the patients at presentation was 66.4 years (range 53-82 years). Furthermore, SOC with coinciding EIC only present in an endometrial polyp was identified in 1 case (case 6), whereas all patients with both EIC and concordant SOC had metastases to omentum, peritoneum, and/or serosa of the uterus and fallopian tubes.

Table 1: Patient characteristics and pathological findings in serous ovarian cancer patients with complete uterine and tubal examination (*N* = 9).

Case	Age (years)	Ovarian carcinoma histology	Adnexal involvement	EIC present	Uterine LVSI	Endometrial sections evaluated	STIC present
1	59	Serous	Bilateral	Yes	No	3	No
2	53	Serous	Bilateral	Yes	No	10	No
3	62	Serous	Bilateral	Yes*	Yes	7	No
4	66	Serous	Unilateral	Yes	Yes	5	No
5	76	Serous	Unilateral	Yes	No	4	No
6	70	Serous	Bilateral	Yes*,†	No	2	No
7	53	Serous	Bilateral	Yes	No	8	No
8	82	Serous	Unilateral	Yes	Yes	4	No
9	77	Serous	Bilateral	Yes	No	5	No

*EIC: endometrial intraepithelial carcinoma; LVSI: lymphovascular space invasion; STIC: serous tubal intraepithelial carcinoma; *EIC: was micro-invasive in these cases, without myometrial invasion; †EIC: was only present in a polyp.*

Immunohistochemical features

Immunohistochemical findings in both intrauterine EIC and its concordant SOC are summarized in Table 2. Immunostaining for the 4 protein markers (p53, Ki-67, ER, and PR) revealed almost identical expression patterns and similar intensities in each pair of EIC and coincident SOC. In 6 of the 9 cases, both EIC and its concordant SOC showed strong nuclear staining for p53. Interestingly, in the other 3 cases, p53 immunostaining was absent in both EIC and in SOC. The probability of these findings by chance is $p = 0.012$. Percentages of Ki-67 nuclear staining ranged from 5% to 90%, although the percentage of positive staining was almost identical in each pair of EIC and SOC. The same holds true for the steroid hormone receptors (ER and PR); although most cases showed no expression of PR in EIC and SOC, 2 of the 9 cases showed nuclear staining for both; whereas in 6 cases, nuclear expression of ER was found in both coincident lesions. Two representative cases with their immunostaining pattern for both EIC and SOC are presented in Figure 1.

Table 2: Immunohistochemistry characteristics of endometrial intraepithelial carcinoma and its concordant serous ovarian carcinoma (*N* = 9).

	p53		Ki-67 (%)		ER		PR	
Case	EIC	SOC	EIC	SOC	EIC	SOC	EIC	SOC
1	-	-	60%	70%	+	++	-	-
2	-	-	20%	20%	+	+	+	+
3	+++	+++	30%	50%	+	++	-	-
4	+++	+++	80%	80%	+	-	-	-
5	+++	+++	5%	5%	-	-	-	-
6	-	-	50%	70%	+++	+++	-	-
7	+++	+++	30%	30%	-	-	-	-
8	+++	+++	70%	90%	+	++	-	-
9	+++	+++	60%	80%	++	++	+	+

EIC: endometrial intraepithelial carcinoma; SOC: serous ovarian carcinoma; ER: estrogen receptor; PR: progesterone receptor. The intensity of staining was scored as; - (negative); + (slight); ++ (moderate); +++ (strong).

TP53 mutations in EIC and SOC

The similarities in p53 protein expression between EIC and coinciding SOC led us to investigate whether this was a result of *TP53* mutations in exons 5 to 8 and whether these mutations were similar in EIC and coinciding SOC. In all 9 cases, we were able to successfully analyze exons 5 to 8 of the *TP53* gene in both the tumor and its concordant EIC. Identical *TP53* mutations were found in both EIC and SOC in 3 of the 9 cases (33%; Table 3: cases 3, 7, and 8), and coincided with high p53 protein expression in these lesions (Table 2). The adjacent normal tissue in these cases did not contain mutated *TP53*, indicating that the mutations were EIC and tumor specific. Although the detected mutations were identical between EIC and the corresponding SOC, the type of mutations between cases was different. In 2 cases (cases 1 and 6), we found a *TP53* mutation in EIC, whereas the corresponding SOC showed a wild-type *TP53* sequence. In the 4 other cases, the sequencing data revealed no mutations in exons 5 to 8 in either the EIC or the ovarian carcinoma.

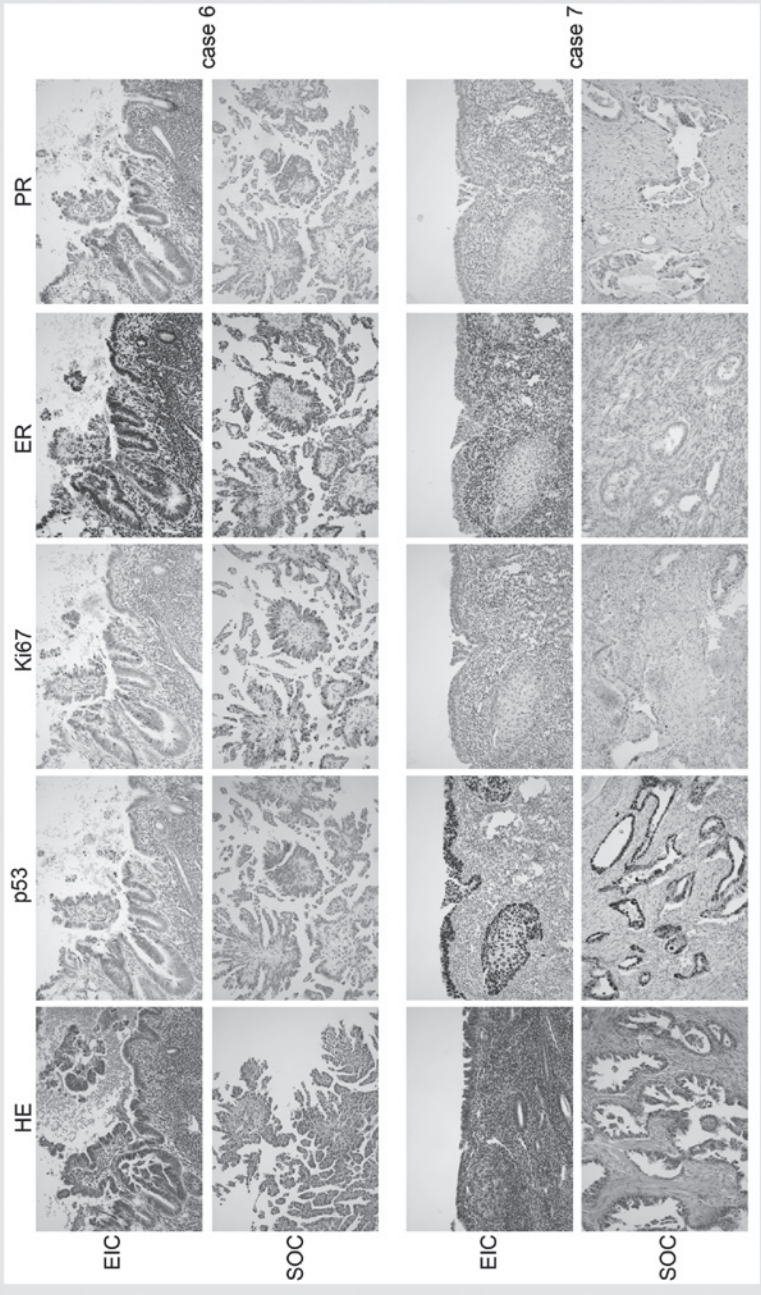

Figure 1: Representative sections of endometrial intraepithelial carcinoma (EIC) and serous ovarian carcinoma (SOC) stained for different proteins by immunohistochemistry. Case numbers correspond to the cases summarized in Tables 1 & 2. HE: hematoxylin-eosin staining; ER: estrogen receptor; PR: progesterone receptor.

Table 3: *TP53* mutation analysis of endometrial intraepithelial carcinoma (EIC) and its concordant serous ovarian carcinoma (SOC).

Case	Tissue	Coding Description	Base change	Codon	Effect	Reported ovarian Carcinoma with this mutation	Total Citations for this mutation
1	**EIC**	**c.878G>A**	**G > A**	**293**	**Missense**	5	51
	Cntr Endometrium	None					
	SOC	None					
	Cntr Ovary	None					
2	EIC	None					
	Cntr Endometrium	None					
	SOC	None					
	Cntr Ovary	None					
3	**EIC**	**c.659A>G**	**A > G**	**220**	**Missense**		
	Cntr Endometrium	None					
	SOC	**c.659A>G**	**A > G**	**220**	**Missense**	50	398
	Cntr Ovary	None					
4	EIC	None					
	Cntr Endometrium	None					
	SOC	None					
	Cntr Ovary	None					
5	EIC	None					
	Cntr Endometrium	None					
	SOC	None					
	Cntr Ovary	None					
6	**EIC**	**c.696C>T**	**C > T**	**232**	**Silent**	2	71
	Cntr Endometrium	None					
	SOC	None					
	Cntr Ovary	None					
7	**EIC**	**c.832C>G**	**C > G**	**278**	**Missense**		
	Cntr Endometrium	None					
	SOC	**c.832C>G**	**C > G**	**278**	**Missense**	20	303
	Cntr Ovary	None					
8	**EIC**	**c.527G>A**	**G > A**	**176**	**Missense**		
	Cntr Endometrium	None					
	SOC	**c.527G>A**	**G > A**	**176**	**Missense**	30	404
	Cntr Ovary	None					
9	EIC	None					
	Cntr Endometrium	None					
	SOC	None					
	Cntr Ovary	None					

EIC: endometrial intraepithelial carcinoma; SOC: serous ovarian carcinoma; Cntr: control tissue as reference; Data adapted (http://www-p53.iarc.fr/).

In situ DNA ploidy analysis

The DNA ploidy status of both EIC and concordant SOC were analyzed for each case. For each patient, 3 microscopic fields were measured for each lesion (EIC and SOC), with a mean of 57 nuclei measured per field (range 19-115). Both the percentage of aneuploid cells and the degree of aneuploidy were calculated. The degree of aneuploidy was expressed as the mean amount of DNA per cell (i.e. DI) compared to normal diploid endometrial and ovarian control tissue in nonproliferating Ki-67-negative cells. DNA ploidy analyses of individual nuclei demonstrated an increase in the number of aneuploid nuclei in 8 of the 9 SOCs compared to their corresponding EIC (p = 0.039; Figure 2). In addition, the DI per nucleus in SOC was higher (i.e. more aneuploid) compared to EIC (in 8 of 9 cases; p = 0.039). The combined probability of these findings by chance is p = 0.0015. When EIC contained aneuploid cells, the DNA content per cell in the associated tumor was at least comparable or higher. In one case (case 9; Figure 2), the EIC contained more aneuploid cells with a higher DNA index compared to its coinciding ovarian tumor.

DISCUSSION

The pathogenesis of SOC in women has been subject to extensive research and controversy. The traditional view holds that ovarian cancer arises from Müllerian epithelium on the ovarian surface or from intracortical inclusion cysts; however, evidence at the clinical, histopathological, or DNA level is lacking to prove this concept. Here, we provide support for our previously described hypothesis[10] that EIC is a likely precursor lesion for SOC based on immunohistochemical staining patterns, *TP53* mutation, and DNA ploidy analyses.

Our immunohistochemistry data provide first evidence for a possible relation between EIC and coinciding SOC. We found that immunostainings for 4 proteins (p53, MIB-1, ER, and PR) revealed almost identical staining patterns and similar intensities for each pair of EIC and corresponding SOC. These findings are concordant with recently published data in which similar expression profiles were shown between EIC and extra-uterine deposits in the ovaries, fallopian tube, omentum, or peritoneum.[22,26,27] Our histopathologic examination and immunohistochemical data alone cannot unequivocally distinguish between a monoclonal or multicentric origin. EIC in the uterine cavity, although without myometrial invasion, can be associated with extensive extra-uterine carcinomatosis.[26,28-30]

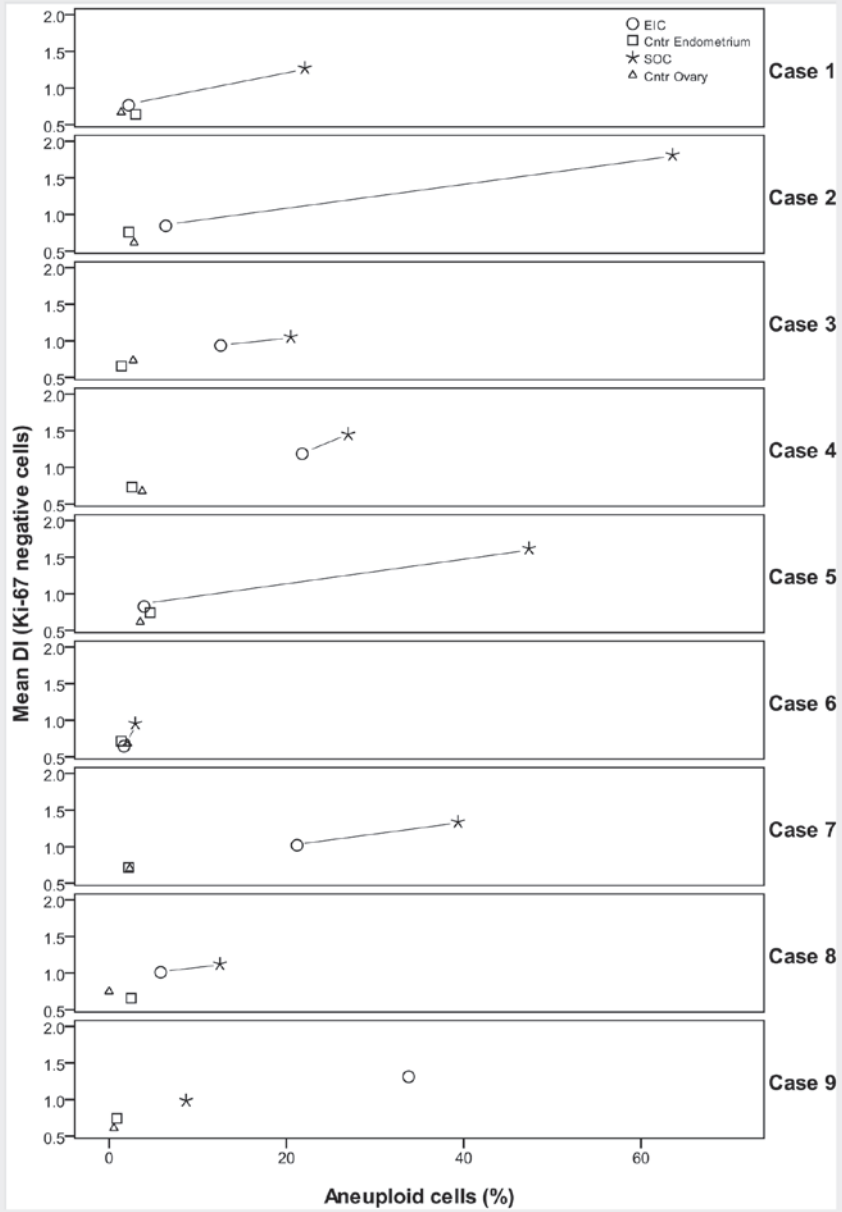

Figure 2: Scatterplot of *in situ* DNA ploidy analysis of endometrial intraepithelial carcinoma (EIC) and its concordant serous ovarian carcinoma (SOC). X-axis shows the percentage of aneuploid cells in the different tissues, whereas the Y-axis indicates the degree of aneuploidy for each individual tissue type, measured as the total amount of DNA in non-dividing Ki-67 negative cells. Normal diploid endometrial and ovarian tissues were used as reference tissues. Overall, the number of aneuploid cells and the degree of aneuploidy was significantly higher in SOC: compared to EIC: (p = 0.0015). DI: DNA index; cntr: control tissue.

Several theories have been proposed to explain the relationship between intra-uterine disease and extra-uterine disease, including early lymphatic spread and synchronous primary tumors (multicentricity). EIC may present with focal LVSI and lymphovascular metastasis may explain their extra-uterine spread in some cases. However, only 3 of the 9 cases of EIC in this study with concordant SOC showed LVSI. Importantly, EIC can present with extensive peritoneal metastases. Shedding of the (pre)malignant tumor cells into the uterine cavity after which the cells are transported through the fallopian tube lumen onto the ovaries and other pelvic peritoneal surfaces is the most likely mechanism. This is also substantiated by infrequent findings of so-called intransit deposits of serous carcinoma cells in the fallopian tube. Alterations in cell surface adhesion molecule expression, including E-cadherin and β-catenin, is associated with the loosely cohesive nature of these (pre) malignant cells resulting in implantation at distant sites.[31-33]

The most widely accepted model of evolution of primary cancers to metastases is the clonal evolution model in which tumors develop by a process of linear clonal evolution driven by accumulation of somatic genetic alterations. Mutation of the *TP53* tumor suppressor gene has been detected in a diverse array of tumor types and is the most commonly altered gene in human malignancies known to date.[34] Although the *TP53* gene consists of a total of 11 exons, approximately 90% of mutations occur in exons 5 to 8.[34] Studies have already shown a high prevalence of *TP53* mutations in UPSC and its precursor EIC: up to 90% and 80%, respectively.[21,29] Importantly, molecular studies on a small number of cases have recently found identical *TP53* mutations in EIC lesions and their corresponding extra-uterine tumor deposits, suggesting a clonal relationship.[14,21,26] In the present study, we identified identical *TP53* mutations in each pair of EIC and concordant SOC in 3 of the 9 cases (33%), highly suggestive for a clonal origin. In 2 cases, *TP53* mutations were found in EIC and not in their corresponding SOC. However, both mutations have a relatively rare incidence in human tumors and more specifically in ovarian carcinoma (Table 3), and one of the mutations is a silent mutation (case 6) not causing changes in the amino acid sequence of the p53 protein. We speculate that these 2 mutations do not provide a major advantage for the malignant progression of EIC toward SOC. In 4 other cases, mutation analysis revealed no *TP53* mutations in exons 5 to 8 in EIC or SOC. In addition, we expected to find *TP53* mutations in those cases with immunohistochemically negative staining, most likely based on frameshift mutations. However, although approximately 90% of human *TP53* mutations occur in exons 5 to 8, we cannot rule out the possibility of p53 function altering mutations within other exons of the *TP53* gene, which were not investigated in this study.

It can be argued that EIC found in the endometrium represents intraepithelial spread from serous peritoneal or ovarian carcinomas. This cannot entirely be refuted on purely morphologic and immunohistochemical characteristics. DNA aneuploidy, an aberrant chromosome number, has been suggested as a useful marker for neoplastic progression of premalignant lesions at different localizations, including esophagus, skin, head and neck, and colon.[35,36] It has been demonstrated previously that most of ovarian carcinomas are aneuploid.[37,38] Our finding of a greater-than-diploid DNA content in nondividing epithelial cells in both EIC and SOC indicates aneuploidy in both lesions.

More importantly, the severity of DNA aneuploidy in SOC was higher compared to the associated EIC in 8 of the 9 cases, suggesting an accumulation of DNA aberration.

In conclusion, it should be emphasized that the data of this study are presented as a first indication for EIC as a possible precursor lesion for SOC, based on a retrospective analysis of the endometrium with a limited number of samples and markers. In contrast, the view of ovarian carcinogenesis has been challenged lately through evidence that SOC arises in the fimbriae of the fallopian tube.[39,40] This most widely accepted concept at present originated with the discovery of STIC in the fimbriae of BRCA mutation carriers. Subsequently, STICs were reported in women with ovarian or peritoneal serous carcinomas, regardless of family history.[41] Based on our findings, we propose that EIC is another credible source of SOC along with fallopian tube precursors. Total analysis of the endometrium has not been part of the routine workup in patients with ovarian cancer. In most cases, limited tissue blocks of the endometrium were available or only a minimal fragment was archived. Therefore, we expect that the incidence of coexisting EIC and SOC that we report in this study is a significant underestimation. Only a prospective in-depth analysis of the total endometrium will reveal its true incidence. Despite this pitfall of our study, our data support a new concept of the origin of SOC. With the endometrium as a possible origin of SOC, it is not difficult to understand the preventive effect of tubal ligation and hysterectomy. In addition, all mechanisms that lead to "incessant" ovulation (pregnancy, breast feeding, and oral contraceptives) also have a clear impact on the endometrium and may thus interfere with the development of intrauterine premalignancies. Although the significance of the endometrium as a source of pelvic and ovarian serous carcinoma has to be further validated in a preferably larger longitudinal prospective study, these data could have clinical implications for ovarian cancer management in the future, as its precursor may reside in the endometrium.

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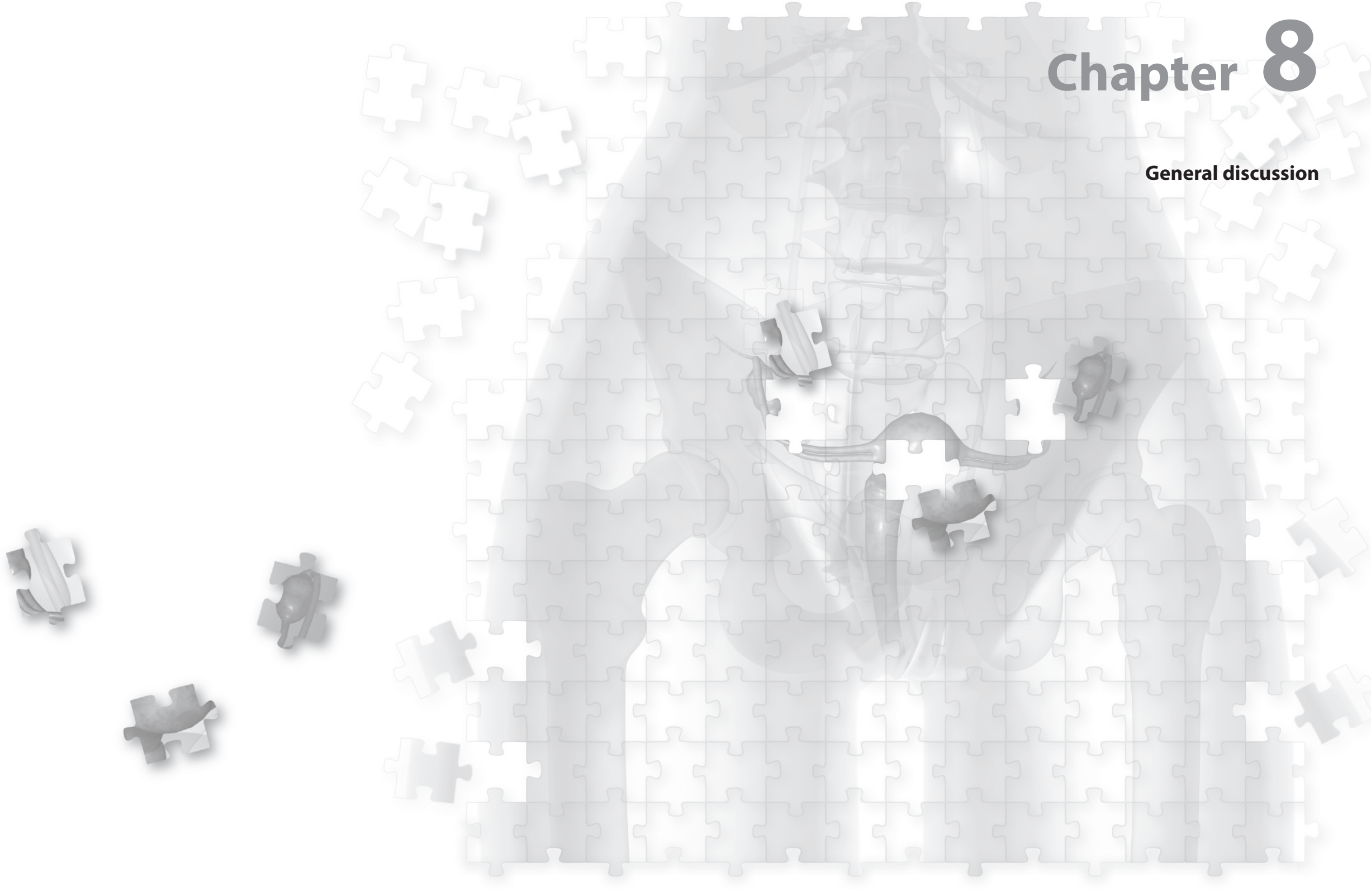
Chapter 8

General discussion



Uterine papillary serous carcinoma (UPSC) is an aggressive variant of endometrial cancer. It has a propensity to metastasize throughout the abdomen, similar to serous carcinoma of the ovary. At present, most clinicians are unfamiliar with the clinical aspects and management of UPSC. Unfortunately, to date little prospective evidence exists regarding how best to treat this subset of patients.

THE NEED FOR A PROSPECTIVE CLINICAL TRIAL

In this thesis, we performed a review of literature to summarize the latest results of various clinical management options in the different sub-stages of UPSC (Chapter 2). Comprehensive surgical staging with maximal cytoreduction seemed to be essential for optimal management. In general, adjuvant radiation therapy is recommended to treat patients with endometrial carcinoma at high risk for recurrent disease. However, external radiation therapy seemed not to be of any significant value for survival in UPSC patients. Brachytherapy improved local control, whereas chemotherapy showed to be beneficial in all (sub)stages of UPSC reducing local and distant recurrences.

Most data on the clinical management of UPSC patients are based on small, retrospective, single institution studies that are plagued by the usual biases and confounders. A proper prospective trial specifically including UPSC patients should be performed to determine the optimal adjuvant treatment strategy. At present, only a very few prospective trials have started to address this question (*www.clinicaltrials.gov*). Most prospective studies to date are non-randomized, only investigating the combination of pelvic radiation and adjuvant chemotherapy in a single arm setting, or testing a new biological in a fase-I or fase-II trial. Intriguingly, some randomized prospective trials do exist testing the efficacy of external pelvic radiation therapy, with or without the addition of combination chemotherapy using cisplatin and paclitaxel. However, in these trials, all subtypes of high-grade endometrial cancer patients or recurrent endometrial cancer patients are lumped together and randomized. Despite the fact that also UPSC patients will be included in these prospective clinical trials, we argue that these studies will not reveal the optimal adjuvant treatment strategy specifically for UPSC patients. The number of included UPSC patients will be relatively low due to its rarity, resulting in a lack of power to perform statistical analysis in both treatment arms on survival outcome specifically for this group. Furthermore, in these studies a comprehensive staging procedure is not mandatory for inclusion of endometrial carcinoma patients. This may undermine the effect of adjuvant therapy specifically in the UPSC group.

Therefore we feel there is an urgent need for a prospective randomized clinical trial specifically for UPSC patients. To be adequately powered, international collaboration will be needed. In such a trial, inclusion criteria are a comprehensive surgical staging procedure with maximum cytoreduction, and UPSC histology (both pure and mixed) confirmed on final uterine specimen. Patients are randomized into two arms of adjuvant therapy: 1) standard treatment (observation only or pelvic radiation therapy with/without brachytherapy, depending on stage of disease),

compared to 2) pelvic radiation with/without brachytherapy and combination chemotherapy (in all stages of UPSC disease, including Stage IA). Although a prospective study with three arms would be even more informative (standard treatment versus combination chemotherapy versus radiation and combination chemotherapy), the number of patients with UPSC needed for this study to be adequately powered might not be feasible. Pending such a randomized controlled trial, we propose an algorithm which can be useful in the clinical management of UPSC patients (Figure 1).

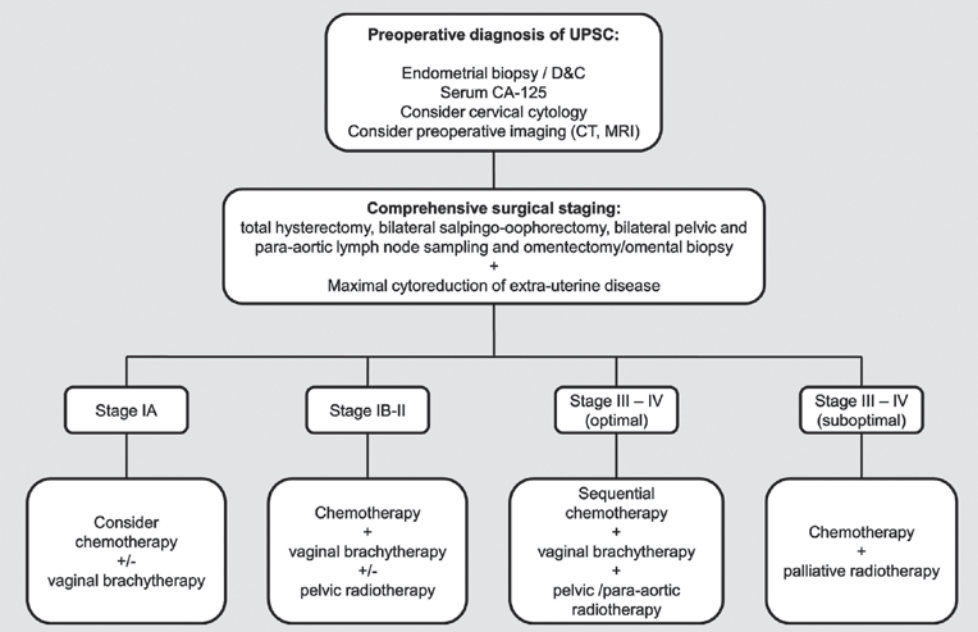


Figure 1: UPSC management algorithm (adapted from Boruta *et al.*[1]).

DIFFICULTIES FOR THE GYNECOLOGIST REGARDING UPSC

To date, it remains difficult to predict extra-uterine disease, recurrence risk, and survival in a UPSC population. Traditional risk factors in endometrial carcinoma patients associated with recurrence and survival, such as myometrial invasion, lymphovascular space invasion (LVSI), age at diagnosis, tumor size, and DNA aneuploidy, were not associated with recurrence risk and survival in UPSC patients.[2-6] In this thesis, we substantiated these findings even more (Chapter 3, Chapter 4, and Chapter 5). We and others were able to show that preoperatively elevated serum CA-125 is an independent predictor for the presence of extra-uterine disease and an independent risk factor for survival in UPSC patients (Chapter 3).[7, 8] Serum CA-125 might have potential clinical application in the management of UPSC patients, although sensitivity and specificity ranged from 78-100%.

More importantly, negative predictive values between 52-74% have been reported, obviously too low to consider a UPSC patient not having extra-uterine disease solely based on preoperatively non-elevated serum CA-125. In addition, others showed that preoperative CA-125 levels were not predictive of complete or suboptimal cytoreduction, and may not predict recurrence in the absence of other clinical findings.[9, 10]

Because of the particularly troublesome feature of UPSC to predict extra-uterine spread of disease preoperatively, imaging might be considered prior to a surgical comprehensive staging procedure. However, the sensitivity of preoperative magnetic resonance (MR) imaging or computed tomography (CT) for diagnosis of lymph node metastases or extra-uterine disease in endometrial carcinoma was only 50-62%, with a specificity of 92-95%.[11-13] The high number of false-negatives on cross-sectional imaging was explained by the high percentage of positive nodes measuring less than 10mm in diameter at pathological examination. Since microscopic metastases are below the detection limits of cross-sectional imaging modalities, both MR imaging and CT seem to have a limited value in the preoperative assessment of extra-uterine disease in UPSC patients.

There is an urgent need for other preoperative tests or tumor biomarkers that can be used to assess disease status and/or that can reliably discriminate between early and advanced stages of UPSC. Investigators have been searching for other markers that can be used either alone or in combination with serum CA-125. One of the novel potential serum markers includes HE4, a soluble mesothelin-related peptide. This HE4 was elevated in all stages of endometrial cancer and was more sensitive in the detection of extra-uterine disease compared to serum CA-125.[14, 15] Another potential marker might be YKL-40, a secreted glycoprotein. It was frequently elevated in all stages of endometrial cancer and may identify high-risk subsets of patients.[16] Combining YKL-40 and CA-125 measurements improved the sensitivity and specificity for detecting advanced stage of disease even more. However, to date none of these markers has been adequately evaluated in women with UPSC.

DIFFICULTIES FOR THE PATHOLOGIST REGARDING UPSC

Pathological examination has been the cornerstone for the diagnosis of UPSC, most often using endometrial biopsy or Dilatation & Curettage (D&C) specimen from the outpatient clinic. UPSC is known to arise in a background of atrophic/weakly proliferative endometrium or in endometrial polyps, often displays considerable morphologic heterogeneity, all associated with a reduced accuracy of the endometrial sampling.[17, 18] In addition, in mixed UPSC cases distinction of serous histology from endometrioid histology may be challenging, with up to 20% of the diagnoses of histological type being altered after surgery.[19-21] To put this into perspective, within our cohort of UPSC patients, preoperative endometrial biopsy or D&C specimen were available in 105 of 115 cases. Without review of these histopathological slides, only in 54 of 105 cases (51.4%) UPSC was diagnosed preoperatively (data not shown). To note, 34 of the remaining 51 cases (66.7%) involved

mixed UPSC cases. Incorrect preoperative diagnosis has potential consequences, since UPSC patients should be treated differently and more aggressively compared to their more common endometrioid endometrial carcinoma (EEC) counterpart. In a small D&C or biopsy specimen of a high-grade carcinoma, we suggest it might be sufficient to indicate that a serous component cannot be excluded, which might encourage the gynecologist to perform a comprehensive staging procedure. This also illustrates the need for more specific tools or biomarkers to identify UPSC more adequately.

Although morphology will always remain the cornerstone in the evaluation of serous tumors (combined with clinical history, appropriate gross evaluation, and sampling), immunohistochemistry can be helpful, if interpreted in the right context, in establishing the correct diagnosis.[22-24] This could be especially important for endometrial biopsy or D&C specimen in which the diagnosis remains inconclusive based on morphological criteria. At present, little is known about the biomarker profile and molecular pathogenesis of UPSC. Most commonly UPSC are hormone (estrogen and progesterone) receptor negative, have elevated Ki-67 proliferation indices, and show mutations in the *TP53* tumor suppressor gene in up to 75-90% of the cases.[25-27] In contrast, the more common EEC frequently display microsatellite instability, preservation of estrogen receptor (ER) and progesterone receptor (PR) status, and mutations in the *PTEN* and *KRAS* genes.[28, 29] However, overlapping molecular profiles have been found in which poorly differentiated EEC lose hormonal receptors status and gain *TP53* gene mutations, whereas UPSC retained hormone receptor expression.[1, 26, 28] These overlapping profiles can make accurate diagnosis even more difficult, especially in very poorly differentiated tumors and mixed UPSC.

Recently, investigators have been focusing on promoter methylation specifically in endometrial carcinoma, since this is a cancer type-specific epigenetic event that plays an important role in tumor development. It was shown that promoter methylation was more common in EEC than UPSC cases, and 90% of EEC and 70% of UPSC cases could be predicted using two promoter loci.[30] Possibly a panel of methylation biomarkers could be useful to distinguish between these two subtypes of endometrial cancer in the near future. Furthermore, L1-CAM as a novel marker for endometrial and ovarian carcinomas was described.[31] While L1-CAM staining was always positive in UPSC, EEC cases were negative.[31, 32] Although the majority of mixed UPSC may be correctly diagnosed on routine H&E stained slides, cases with tiny foci of serous or clear cell types, or with large undifferentiated areas, are easily misdiagnosed. As mixed UPSC represents a large proportion of cases (Chapter 5), L1-CAM staining might be very helpful for the detection of a serous component, allowing a conclusive diagnosis and avoiding false classification.

Although many investigators to date are searching for a highly sensitive and specific biomarker for the identification of UPSC histology, such a marker has not been identified yet. Despite the attenuation of immunophenotypic differences between serous and poorly differentiated EEC, most studies found that a panel of antibodies, including ER, PR, p53, p16, PTEN, and Ki-67, is useful in separating these two subtypes of endometrial carcinoma.[33-35]

In the search for other preoperative tools to identify UPSC more adequately, an interesting finding was the high frequency of atypical or malignant endometrial cells in preoperative cervical cytology of UPSC patients (Chapter 4). Cervical cytology is routinely performed in patients with abnormal vaginal bleeding to exclude cervical pathology. Although cervical cytology appeared to be a poor screening tool for endometrial carcinoma because of its low sensitivity[36], normal or atypical endometrial cells found in cervical cytology of postmenopausal women were predictive for endometrial pathology, cervical stroma involvement and lymph node metastases.[37-39] We found that 87.5% of UPSC patients had abnormal cervical cytology specific for endometrial pathology, and abnormal cytology was associated with extra-uterine disease (Chapter 4). This high frequency most probably is due to the papillary architecture of UPSC and the propensity to exfoliate, although the molecular biology to account for this observation has not been thoroughly investigated yet.[40, 41] More prospective research should be performed, though preoperative abnormal cervical cytology might give an indication to suspect a more aggressive uterine tumor. To further substantiate this, especially when endometrial biopsy or the D&C specimen is inconclusive, immunohistochemistry on cytology might be considered to identify high-grade malignant cells, e.g. serous, clear cell, or undifferentiated cells, from the more common endometrioid or squamous cells. We performed a pilot study using Ki-67 and p53 on cervical cytology as to differentiate between EEC and UPSC, however results were disappointing.

THE METASTATIC POTENTIAL OF UPSC AND ITS PRECURSOR

UPSC is an aggressive form of endometrial carcinoma with a dismal prognosis. UPSC has a very close resemblance with serous ovarian carcinoma (SOC), fallopian tube cancer and other types of pelvic serous cancers. The malignant cell type is morphologically identical for all of these types of cancer and the dissemination of disease and its prognosis is very similar. Several studies have reported on the metastatic properties of UPSC, with a high incidence of pelvic (41,9%) and para-aortic (43,3%) lymph node metastases and omental involvement (15-35%).[42, 43] Typically, 55-87% of the patients have extra-uterine spread of disease at the time of diagnosis.[43-45] The mechanisms proposed to explain the characteristic widespread intra-abdominal dissemination of UPSC include transtubal expulsion of malignant cells into the peritoneal cavity[46, 47], vascular/lymphatic invasion[47, 48], and multifocal disease.[47, 49-51] Although the molecular biology to account for these observations have not been thoroughly investigated, there might be a relation with change of expression of proteins such as integrin, e-cadherin, β-catenin and L1-CAM. Typically, UPSC demonstrates genetic instability at the chromosome level, resulting in a high level of aneuploidy. The proportion of non-diploid karyotypes in UPSC ranged from 70-95%.[52, 53] One of the primary genetic defects is believed to be mutation of the *TP53* tumor suppressor gene, observed in 75-90% of tumors.[54] The expression of the protein E-cadherin, generally considered as a suppressor of tumor progression, has been found to be reduced in UPSC cases. Down-regulation is believed to be a critical step in the epithelial-

mesenchymal transition (EMT).[55, 56] During metastatic progression, polarized epithelial tumor cells undergo a transition into motile mesenchymal cells, allowing them to invade the basement membrane, enter the blood vessels and disseminate to secondary organs. L1-CAM is highly expressed in UPSC cases, with emphasis on the leading edge of the tumor.[32] L1-CAM leads to down-regulation of ER/PR and E-cadherin, which is associated with malignant transformation. In addition, up-regulation of L1-CAM was associated with enhanced cell invasion, and enhances cell motility by up-regulation of integrin expression.[57, 58] Therefore L1-CAM is an important component in serous tumor progression. Furthermore, expression of β-catenin, which plays a role in cell-cell adhesion, intracellular signaling, and maintenance of tissue architecture, is down-regulated in UPSC.[59] Possibly decreased epithelial cell-cell adhesion contributes to the typical capability of serous tumor cells to exfoliate from the primary tumor and disseminate distantly.

Although the carcinogenesis of UPSC is largely unknown, EIC has been described as the non-invasive precursor of UPSC.[60] It was often multifocal, found adjacent to UPSC in >80% of cases (Chapter 5), and exhibits p53 mutations and cytologic features similar to that observed in UPSC.[61, 62] Loss of heterozygosity (LOH) at the *TP53* locus is consistently identified in UPSC, whereas most EIC contained only one mutated *TP53* allele.[27] Furthermore, concordant mutations were identified among EIC and UPSC lesions, and these observations suggest that UPSC develops from EIC and that *TP53* mutation is an early change in tumor progression.[27] EIC has rarely been reported as the initial diagnostic manifestation of non-invasive UPSC.[61] Markedly, EIC without invasive carcinoma within the uterine corpus was found coinciding with metastases on ovaries, fallopian tube or peritoneum.[62-65] Although lymphovascular metastasis may explain the extra-uterine spread of EIC in some cases, only a very few cases of EIC itself showed LVSI (Chapter 5). The loosely cohesive nature of these cells within EIC lesions, primarily based on similar changes in expression of cell-cell adhesion proteins as in UPSC, predisposes them to shed easily. Shedding of the (pre)malignant tumor cells into the uterine cavity after which the cells are transported through the fallopian tube lumen onto the ovaries and other pelvic peritoneal surfaces is the most likely mechanism. Baergen and colleagues were the first to show that EIC and concordant extra-uterine serous lesion were of clonal origin, based on *TP53* mutation analysis.[66] In addition, identical *TP53* mutations in noninvasive serous endometrial lesion or EIC and associated extra-uterine serous carcinoma deposits have been recently described.[27, 67] Interestingly, in patients with bilateral ovarian cancer the genetic change in the *TP53* gene was identical in both ovarian tumors, highly speculative for a site of origin elsewhere.[67] Furthermore, the *TP53* data discussed above are inconsistent with the interpretation that EIC and extra-uterine serous carcinoma represent independent primaries. In contradiction to the multicentricity theory, the *TP53* gene mutation profiles of EIC or minimal invasive serous carcinoma were identical to those of their corresponding extra-uterine tumor deposits, consistent with a clonal, metastatic process.[27, 64, 66, 67]

CLINICAL MANAGEMENT OF EIC

In contrast to the usual behavior of conventional carcinoma *in situ* of other organs, patients with EIC may present with extra-uterine tumor spread in up to 45% of cases, leading to an advanced stage and a clinical prognosis similar to that of full-blown UPSC.[63, 64] The inability to detect EIC preceding UPSC suggests that EIC acquires invasive and metastatic properties early in its development.[68] For this reason, there is some controversy whether EIC should be classified as true precursor lesion. Such concern is further exacerbated by the fact that these patients without myometrial or lymphovascular invasion have an excellent prognosis if extra-uterine disease is excluded by staging. In contrast, the prognosis is poor if even minimal microscopic extra-uterine disease is found.[63, 64] Meticulous staging is essential for EIC because the prognosis is excellent, even without adjuvant therapy, if extra-uterine disease can be excluded. Clinically, we therefore feel it's prudent that a complete comprehensive staging procedure is performed, even if the lesion is limited to a polyp.

LESION OF ORIGIN FOR SEROUS OVARIAN CARCINOMA

The pathogenesis of serous ovarian carcinoma (SOC) in women has been subject to extensive research and controversy. The traditional view holds that ovarian cancer arises from Müllerian epithelium on the ovarian surface or from intracortical inclusion cysts, but evidence at the clinical, histopathological or DNA level has been lacking to prove this concept. In this thesis, we provided data as a first indication for EIC as a possible precursor lesion for SOC (Chapter 7). Analogous to the theory for the development of endometriosis, transtubal migration of the loosely cohesive cells from EIC foci in the endometrium may be the basis for the development of any type of intraperitoneal serous carcinoma, including SOC. Furthermore, implantation in the fallopian tube may explain part of the so-called in-transit deposits of serous carcinoma infrequently detected by several investigators in the fimbriated end of the tubes. In addition, cases of non-invasive serous lesions or EIC in the endometrium with peritoneal serous metastases have been described coinciding with STIC in the distal end of the fallopian tube.[69] Immunohistochemical staining patterns were identical between EIC, STIC, and intraperitoneal serous lesions, and *TP53* mutation analyses identified shared mutations at all sites.[69] At present, whether EIC and STIC are truly related is far from clear. When multiple serous tumor sites are identified, specifically ovarian and tubal, the role of EIC or STIC as an initiating point, though attractive, is still controversial.[69, 70]

Since most of ovarian carcinoma that are identified in BRCA mutations carriers are SOC, examination of prophylactic salpingo-oophorectomy specimens should theoretically reveal a precursor lesion in at least a subset of these patients. Recent studies of women with hereditary mutations in the BRCA1 and BRCA2 genes have reported detecting early carcinoma in the fallopian tube in a significant percentage of cases; the frequency has varied though between 1-8% (Chapter 6).[71-73] These lesions are typically identified in the distal portion of the fallopian tube, as a noninvasive carcinoma (STIC). Others have now showed that 35-70% of sporadic (nonhereditary) SOC or serous peritoneal

carcinoma classified as ovarian primaries showed mucosal tubal involvement and were associated with STIC.[74] STIC frequently up-regulate oncogene products that are also overexpressed by SOC, and have relatively shorter telomeres compared with concurrent SOC.[75, 76] Furthermore, STIC and SOC were clonally related as the same *TP53* mutations were detected in both STIC and SOC.[77-79] Finally, a gene profiling study showed that serous carcinoma from the fallopian tube and ovary were indistinguishable, and the expression profile of SOC was more closely related to the fallopian tube than to ovarian surface epithelium.[80] These studies suggest strongly that the distal fallopian tube is an important site for serous carcinogenesis. In addition, more and more investigators are now reporting on STIC being identified in a significant portion of serous cancers conventionally classified as primary peritoneal.[74, 77, 81] The evidence developed over the last 5 years supporting a tubal origin for SOC is now much stronger than the cumulative data of several decades investigating ovarian inclusion cysts as the site of origin. Although it is likely that both tubal and non-tubal sites of origin for SOC occur, the fallopian tube appears to be more common.

FUTURE PERSPECTIVES

The findings in this thesis offer new possibilities for further research. First, our results demonstrate that patients with mixed UPSC have a favorable prognosis and survival outcome compared to pure UPSC, in contrast to previously published data.[3, 82, 83] Despite the better prognosis for mixed UPSC patients, we do suggest any endometrial carcinoma with serous differentiation must still be regarded as having high metastatic potential and should be surgically staged. At present, the pathogenesis of mixed UPSC remains unclear. The precursor lesion EIC was equally found among pure and mixed UPSC cases, suggestive for its role in the carcinogenesis of both types of serous endometrial carcinoma. Interestingly, the non-neoplastic endometrium was predominantly athrophic/weakly proliferative in pure UPSC cases compared to more hyperplastic with atypia in mixed UPSC cases. Since atypical hyperplasia is the well know precursor lesion for EEC, it would be interesting to study the role of atypical hyperplasia in the carcinogenesis of mixed UPSC. Immunohistochemical and genetic analyses might give further insights whether the endometrioid component within mixed UPSC cases originates from atypical hyperplastic lesions, or whether it is the result of dedifferentiation within the tumor environment.

Second, another and intriguing question remains the origin of serous ovarian carcinoma (SOC). More and more data is now accumulating that STIC in the fallopian tube is the precursor lesion for most serous ovarian and pelvic carcinoma. However, this site of origin remains controversial, since epidemiological studies show that tubal ligation and hysterectomy are reducing the life-time risk for ovarian cancer by up to 50%. To note, in patients receiving one of these interventions, the ovaries and fallopian tubes remain *in situ*. Furthermore, a significant subset of SOC and serous peritoneal carcinoma are not associated with STIC.

In this thesis, we proposed that EIC is another possible source of SOC along with the fallopian tube

precursor (STIC). In addition, all mechanisms that lead to 'incessant' ovulation (pregnancy, breast feeding and oral contraceptives) also have a clear impact on the endometrium and may thus interfere with the development of intrauterine premalignancies. The number of serous ovarian or peritoneal carcinoma cases which truly reflect a distal fallopian tube (STIC) or endometrial (EIC) origin remains to be determined by much larger studies. In addition, in most recent retrospective studies the fallopian tubes were not embedded *in toto* and serially sectioned using the SEE-FIM protocol, likely resulting in an underestimation of the incidence of STIC in the distal tube. Furthermore, total histopathological review of the endometrium has not been part of the routine workup in serous ovarian and pelvic cancer patients, and limited amount of endometrium was sampled. Thus, the results of previous studies and of this thesis stress the importance of conducting a proper prospective in depth analysis of both the fallopian tubes and total endometrium of all serous ovarian and pelvic carcinoma patients to permit discovery of early serous cancers. Subsequently, immunohistochemical and genetic analyses should be performed to provide evidence for clonality among precursor lesions identified in the endometrium or fallopian tube and the concurrent full-blown serous carcinoma.

If the fallopian tube is considered the site of origin for most SOC and serous peritoneal carcinoma, then preventive strategies become much more realistic.[84, 85] Sparing the fallopian tubes during hysterectomy for benign uterine indications provides no clear physiological benefit, as the remaining tubes are completely devoid of any function in the aftermath of such a procedure, and the patients hormone profile is not altered by salpingectomy.[86] If adnexal SOC are unequivocally shown to develop almost exclusively in the fimbria, salpingectomy alone would be sufficient to reduce the risk of serous ovarian and pelvic cancer while preserving ovarian function. Thus, an interesting prospective trial would be to include BRCA mutations carriers at risk for serous carcinoma, and to randomize these patients into two study arms: 1) perform a complete salpingo-oophorectomy as standard of care, or 2) perform a salpingectomy only. This study would need a long term follow-up comparing the prevalence of serous ovarian and pelvic cancers. Such a study may shed light on the hypothesis that SOC truly is not originating from the ovaries. However, whether SOC and serous peritoneal carcinoma have an endometrial or tubal origin still remains a question.

Ultimately, we believe SOC might not be ovarian in origin but rather is secondary. Characterization of putative precursor lesions is fundamental in elucidating the molecular pathogenesis of cancer and has profound implications for early detection, prevention and treatment.

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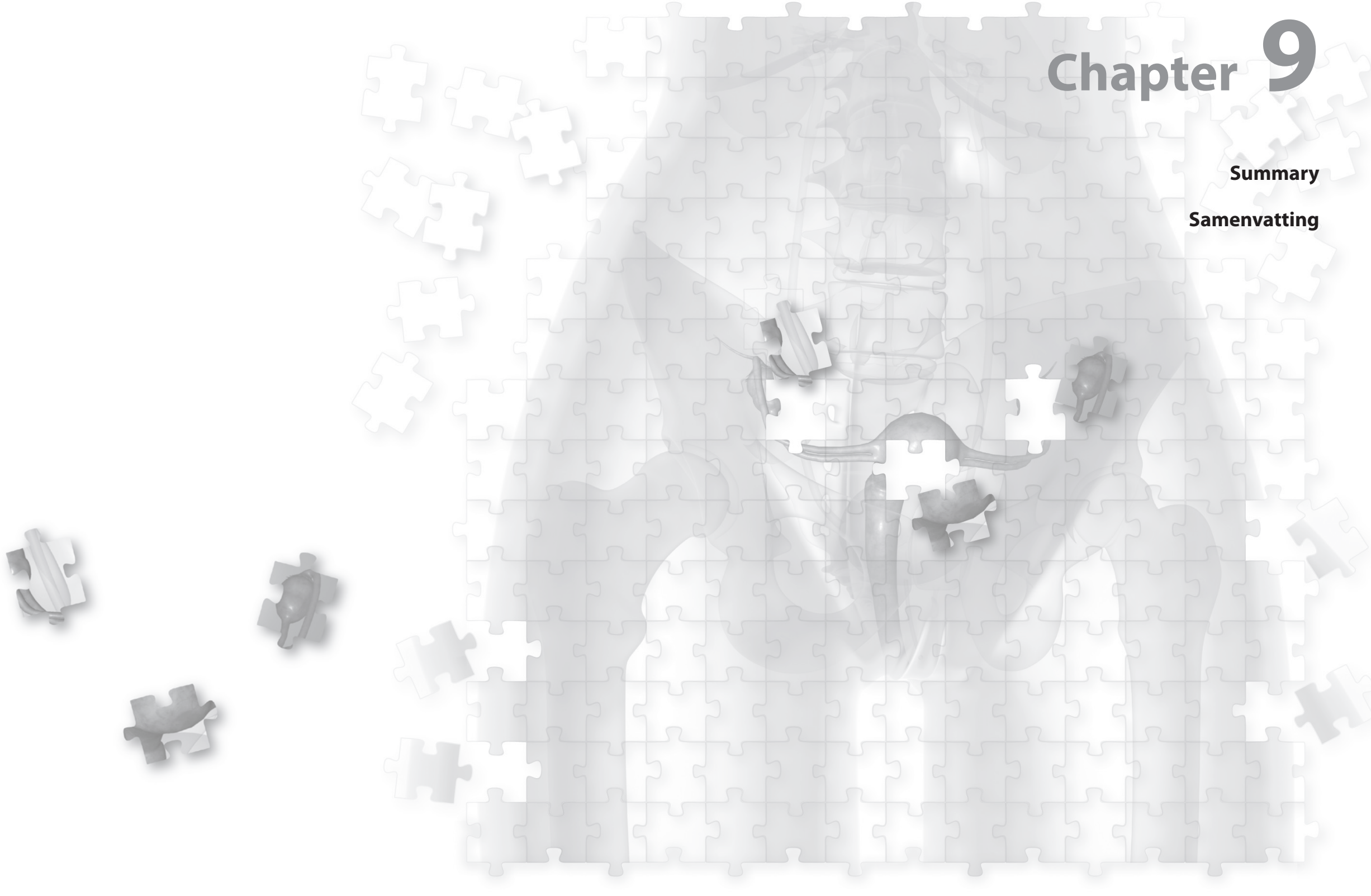
Chapter 9



SUMMARY

Uterine papillary serous carcinoma (UPSC) is a highly aggressive subtype of endometrial cancer that is disproportionately responsible for up to 40% of all endometrial cancer-related deaths. UPSC resembles serous ovarian carcinoma (SOC) morphologically and also in its clinical behavior, characterized by high recurrence and mortality rates even in early stage of disease. Improving the care for patients with UPSC requires further understanding of the etiology and clinical behavior of the disease. **Chapter 1** reviewed the history, epidemiology, clinical characteristics, preoperative assessment options, treatment modalities, and pathogenesis of UPSC and its precursor endometrial intraepithelial carcinoma (EIC) as an introduction to the studies performed and described in this thesis.

Due to its rarity, most clinicians are unfamiliar with the clinical aspects and management of patients with UPSC. Furthermore, little prospective evidence exists regarding how best to treat this subset of patients with endometrial cancer. Pending proper prospective randomized trials, in **chapter 2** we performed a review on the clinical aspects of UPSC with a focus on the effects of staging and adjuvant therapy on recurrence rate and survival outcome in the different (sub-) stages of UPSC. We found that the necessity of comprehensive surgical staging in patients with UPSC cannot be overemphasized, since many studies have demonstrated the propensity of UPSC for extra-uterine spread. Therefore, comprehensive surgical staging of all UPSC patients should be recommended, regardless of the clinical stage at presentation. Furthermore, maximum cytoreduction enhanced response to adjuvant therapy and provided a favorable survival rate. With respect to adjuvant therapy, external radiation therapy seemed not to be of any significant value for survival. Brachytherapy showed to be of minor importance for overall survival, although it did improve local control and progression free survival and thus should be considered. Chemotherapy showed to be beneficial in all (sub)stages of UPSC, even in stage I disease, reducing local and distant recurrences.

A particularly troublesome feature of UPSC has been the inability to predict extra-uterine spread of disease. A preoperative test or tumor marker that can reliably discriminate between early and advanced stages of UPSC would be helpful in the clinical management and planning, and possibly in prognostication. In **chapter 3** we determined the utility of preoperative serum CA-125 as a predictor of extra-uterine disease and as a prognostic factor for survival in patients diagnosed with UPSC. In patients with extra-uterine disease the preoperative serum CA-125 level was significantly higher (median 124.0 U/mL) compared to patients without extra-uterine disease (median 17.5 U/mL). Preoperative serum CA-125 > 45 U/mL was significantly associated with the presence of extra-uterine disease, and UPSC patients with a preoperative CA-125 level > 45 U/mL had a 6.30 times greater risk for extra-uterine disease. In addition, advanced FIGO stage and preoperative CA-125 > 45 U/mL were associated with both progression free and overall survival. Our results showed that serum CA-125 elevation was predictive for the presence of extra-uterine disease, and preoperative

serum CA-125 was an independent risk factor for survival. Routine measurement of preoperative serum CA-125 might be considered in patients with UPSC.

In asymptomatic women, cervical cytology appeared to be a poor screening tool for endometrial carcinoma because of its low sensitivity. However, when normal or atypical endometrial cells were found in cervical cytology of postmenopausal women, it was predictive for endometrial pathology. In **chapter 4** we evaluated the presence of shedded atypical endometrial cells in cervical cytology of patients with UPSC as compared to patients with EEC. In total, 87.5% UPSC patients had abnormal cervical cytology, specific for endometrial pathology preoperatively, in contrast to 37.8% EEC patients. Extra-uterine disease was associated with abnormal cervical cytology in patients with UPSC, whereas in patients with EEC abnormal cervical cytology was associated with cervical involvement. Abnormal cervical cytology specific for endometrial pathology was not associated with PFS in either UPSC or EEC patients. Our results showed that abnormal cervical cytology was associated with poor prognostic factors, although it cannot be used as prognosticator for survival.

UPSC often displays considerable morphologic heterogeneity, co-existing with at least one other subtype of uterine cancer. It is largely unknown whether the percentage UPSC histology is predictive of recurrence rates or survival. Furthermore, histopathologic studies suggested that the majority of UPSC develop from endometrial intraepithelial carcinoma (EIC). In **chapter 5** we studied whether mixed versus pure UPSC histology affected clinical outcome. Furthermore, we assessed the uninvolved endometrium in both pure and mixed UPSC cases for their association with EIC. We found 50 patient cases (46.3%) with pure UPSC histology, whereas 58 cases (53.7%) had mixed UPSC histology. Together with early stage of disease, mixed UPSC histology was associated with lower recurrence risk and favorable survival compared to pure UPSC. Both progression free and overall survival were significantly shorter in the group of pure UPSC patients compared to mixed UPSC ($p < 0.001$). Atrophic or weakly proliferative endometrium was found in 90.7% of pure UPSC cases, whereas hyperplastic endometrium with atypia was more commonly found in 34.7% of patients with mixed UPSC ($p = 0.004$). Our results showed that pure UPSC histology and FIGO stage were the most important risk factors for recurrence and survival in patients with UPSC. Furthermore, EIC as precursor was equally found in both pure and mixed UPSC cases in 84% of cases, suggestive for its role in the carcinogenesis of both types of serous endometrial carcinoma.

Recently, a predisposition for SOC was discovered in the fallopian tubes of women with a germline BRCA-mutation. Prophylactic bilateral salpingo-oophorectomy (pBSO) is performed in the majority of BRCA-mutation carriers, and incidental findings of occult invasive carcinoma and the precursor lesion tubal intraepithelial carcinoma (STIC) were found. Recent studies provided some compelling evidence that a proportion of primary SOC actually arised from these precursor lesions. In **chapter 6** we set out to identify tubal epithelial lesions in a large cohort of women with BRCA 1/2 mutation

who underwent pBSO, in comparison to a large control group of women that underwent BSO for non-malignant reasons. A total number of 226 women with a BRCA-mutation and 105 controls were included. Of the women with a BRCA-mutation, 6% had a STIC, and 1% had an invasive tubal carcinoma in their BSO specimen, in contrast to no tubal neoplasia (STIC or invasive carcinoma) in controls ($p = 0.004$). In the majority of cases, invasive tubal carcinoma and STIC were identified in the distal fimbria. Our results showed that occult invasive tubal carcinoma and STIC were present in 7% of the BRCA-mutation carriers but were absent in control cases. The fact that invasive carcinomas and STICs were not identified in controls marked their uniqueness for women at high risk for SOC and therefore their suggested role in carcinogenesis.

At present, experimental or histopathological evidence is lacking for an ovarian origin for SOC, and a precursor lesion was never identified in the ovary itself. In **chapter 7** we studied an alternative hypothesis regarding the site of origin for SOC in which endometrial intraepithelial carcinoma (EIC) was proposed to be the precursor lesion. We selected patients with SOC and coinciding EIC in the endometrium. Immunostaining for 4 protein markers (p53, Ki-67, ER, and PR) revealed almost identical expression patterns and similar intensities in each pair of EIC and coincident SOC. Furthermore, identical *TP53* gene mutations were found in both EIC and SOC in 33% of cases. DNA ploidy analyses demonstrated an increase in the number of aneuploid nuclei in 8 of the 9 SOCs compared to their corresponding EIC ($p = 0.039$). In addition, the DNA index per nucleus in SOC was higher (i.e. more aneuploid) compared to EIC ($p = 0.039$). Our results support our hypothesis that EIC is a likely precursor lesion for SOC based on immunohistochemical staining patterns, *TP53* mutation, and DNA ploidy analyses. With the endometrium as a possible origin of SOC, it is not difficult to understand the preventive effect of tubal ligation and hysterectomy.

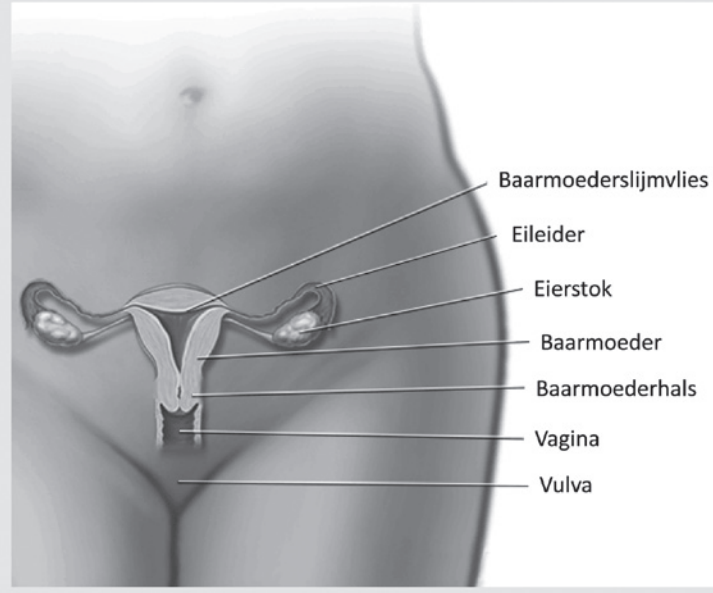
In **chapter 8** we summarized our main findings and discussed our results. We especially stressed the need for a prospective trial on the clinical management of patients with UPSC, and we provided an algorithm which can be useful in the clinical management pending such a trial. Furthermore, we discussed some difficulties regarding UPSC from the gynecologist and pathologist perspective. In addition, we discussed the metastatic potential of UPSC and its precursor EIC, and the possible role EIC and STIC might play in the pathogenesis of SOC. Finally, we postulated some ideas for future research.

SAMENVATTING

Kanker is de huidige doodsoorzaak nummer 1 in Nederland, met in 2010 ruim 42.000 overledenen. De ziekte is daarmee verantwoordelijk voor bijna een derde van de totale sterfte. Door bevolkingsgroei en vergrijzing neemt het aantal sterfgevallen door kanker al decennia geleidelijk toe. Binnen de gynaecologische oncologie, welke zich bezig houdt met kanker van het vrouwelijke voortplantingsstelsel, kennen we kanker van de baarmoederhals, baarmoeder, eileiders, eierstokken, vagina en vulva (omvat schaamlippen, clitoris, uitgang urinebuis, en opening vagina; figuur 1). Hiervan komt baarmoederkanker het meest voor, met ongeveer 1930 nieuwe gevallen per jaar in Nederland en ongeveer 425 sterfgevallen per jaar. Baarmoederkanker presenteert zich vaak met vaginaal bloedverlies gedurende de (post)menopauze. Dit is een alarmsignaal, waardoor in veel gevallen de diagnose reeds in een vroeg stadium kan worden vastgesteld. Meestal is hierbij geen sprake van uitzaaiingen (metastasen) en omvat de standaard behandeling het operatief verwijderen van de baarmoeder en eierstokken, in een aantal gevallen gevolgd door nabehandeling met externe röntgenbestralingen. De prognose van deze patiënten is vaak gunstig, met een gemiddelde 5-jaars overleving van ongeveer 70-80%.

Essentieel bij alle vormen van kanker, dus ook binnen de gynaecologische oncologie, is het stellen van de juiste diagnose. Met andere woorden: om welke soort kanker (oftewel carcinoom) gaat het precies, welk histologisch type? Dit is cruciaal voor de verdere behandeling en prognose van de patiënte. Deze diagnose kan worden gesteld door de patholoog-anatoom. Dit doet hij door relevante stukken weefsel uit het chirurgische preparaat te snijden. Met behulp van een microscoop wordt een histologische diagnose gesteld, op basis van cel-kenmerken, typische afwijkingen en specifieke kleuringen. Het meest vóórkomende histologisch subtype van baarmoederkanker is het endometrioïde type (ongeveer 80% van alle gevallen) en kent een goede prognose.

Hoofdstuk 1 betreft een algemene inleiding op de studies zoals die zijn gedaan en beschreven in dit proefschrift. Binnen de verschillende vormen van baarmoederkanker kennen we een uitermate agressief histologisch subtype: het sereus carcinoom van de baarmoeder (uterus). Ondanks dat dit subtype maar ongeveer 10% van alle baarmoederkanker gevallen per jaar omvat, is het verantwoordelijk voor ongeveer 40% van alle overledenen met baarmoederkanker. Dit komt met name omdat ten tijde van diagnose sereus carcinoom van de uterus meestal al sprake is van vergevorderde ziekte: tussen 55-87% van de patiënten heeft reeds metastasen, met name in de buikholte. Deze patiënten hebben dan ook een uitermate slechte prognose, met een gemiddelde 5-jaars overleving van slechts 18-45%. De standaard behandeling voor baarmoederkanker lijkt tot dusverre dan ook suboptimaal voor dit agressievere subtype. In dit hoofdstuk beschrijven we de mogelijke ontstaanswijze inclusief het voorloperstadium, de epidemiologie, tumorkarakteristieken en behandelingsopties van dit subtype baarmoederkanker. Een beter begrip is essentieel om de behandeling te kunnen verbeteren.



Figuur 1: het vrouwelijke voortplantingsstelsel.

Hoofdstuk 2 betreft een overzicht van de huidige literatuur omtrent het sereus carcinoom van de uterus. Er wordt gefocust op de klinische aspecten en behandeling, met een nadruk op welk type chirurgie en aanvullende therapie het beste kan worden toegepast. Door de relatieve zeldzaamheid zijn de meeste gynaecologen vrij onbekend met dit agressieve subtype baarmoederkanker. Daarnaast zijn op dit moment maar een zeer gelimiteerd aantal studies in de literatuur bekend die een uitspraak doen omtrent de best mogelijke behandeling.

Het sereus carcinoom van de uterus is voor de patholoog-anatoom histologisch identiek aan het sereus carcinoom van de eierstok (ovarium). Ook in klinisch gedrag, potentieel tot metastaseren, prognose en overleving lijken ze sterk op elkaar. De behandeling verschilt echter. Het sereus carcinoom van het ovarium wordt behandeld door toepassing van uitgebreide specialistische chirurgie (debulking chirurgie met stadiëring genaamd), waarbij baarmoeder inclusief baarmoederhals, eierstokken, eileiders, vetschort, lymfeklieren zowel in het kleine bekken als hogerop langs de grote lichaamsslagader (aorta), en alle verdachte laesies zichtbaar ten tijde van de operatie worden weg genomen. Daarbij bestaat de nabehandeling van eierstokkanker bijna altijd uit aanvullende chemotherapie. In internationale literatuur werd recent gesuggereerd dat het sereus carcinoom van de uterus wellicht hetzelfde zou moeten worden behandeld als het sereus carcinoom van het ovarium.

Na analyse van de beschikbare literatuur kunnen we niet sterk genoeg benadrukken dat het zeer belangrijk lijkt om patiënten met een sereus carcinoom van de uterus inderdaad volledig chirurgisch te debulken, inclusief een stadiëring procedure. Het blijkt dat patiënten zonder klinische verdenking

voor gemetastaseerde ziekte toch in 21-42% wel degelijk (micro-) metastasen hebben, ontdekt door een stadiëring procedure. Deze metastasen zijn met name in lymfklieren en in het vetschort gelokaliseerd. Daarnaast is debulking chirurgie (het maximaal weghalen van alle macroscopisch zichtbare kanker) sterk geassocieerd met een verbeterde respons op aanvullende chemotherapie. Patiënten met sereus carcinoom van de uterus, waarbij debulking chirurgie gecombineerd wordt met een stadiëring procedure, hebben een significant betere overleving vergeleken met patiënten die op de conventionele manier chirurgisch worden behandeld.
Ondanks dat de standaard aanvullende behandeling voor baarmoederkanker externe röntgen-bestralingen omvat, blijkt deze aanvullende therapie nauwelijks van waarde bij het sereus carcinoom van de uterus. Er worden nauwelijks verbeteringen gevonden in gezondheid, de kans op hernieuwde ziekte (recidief), en overleving vergeleken met patiënten die niet worden nabehandeld. Daarbij ontstaan er ernstige neveneffecten en complicaties. Brachytherapie wordt ook wel toegepast. Bij deze vorm van therapie wordt een röntgen bestralingsbron vaginaal ingebracht zodat lokale behandeling kan worden gegeven. Deze manier van behandelen kent veel minder neveneffecten en wordt meestal goed verdragen door de patiënte. Ondanks dat het een efficiënte methode is om de kanker lokaal te beteugelen en de kwaliteit van leven te verbeteren, wordt er geen winst geboekt in overleving. Aanvullende chemotherapie blijkt de enige behandeling die significant de overleving van patiënten met sereus carcinoom van de uterus verbeterd, zelfs bij patiënten met een vroeg stadium (zonder metastasen) van de ziekte. Hiermee wordt ook de kans op het krijgen van een lokaal recidief danwel een recidief op afstand veel kleiner. In afwachting van grote prospectieve klinische studies lijkt het dus aan te bevelen om patiënten met sereus carcinoom van de uterus uitgebreid chirurgisch te behandelen met debulking chirurgie inclusief een stadiëring procedure, gevolgd door aanvullende chemotherapie.

In **Hoofdstuk 3** wordt onderzocht of het bepalen van de tumormerkstof CA-125 in het bloed van waarde kan zijn om voor de operatie (pre-operatief) gemetastaseerde ziekte te voorspellen bij patiënten met een sereus carcinoom van de uterus. Het is namelijk vaak moeilijk gebleken om pre-operatief adequaat gemetastaseerde ziekte bij deze patiënten te detecteren, terwijl dit erg belangrijk kan zijn in de planning van de (chirurgische) behandeling en in de informatievoorziening naar de patiënte toe: wat kan zij verwachten, wat is haar prognose?
Sinds de ontdekking in 1983 is de tumormerkstof CA-125 in bloed uitgebreid bestudeerd in relatie tot allerlei ziekten en aandoeningen. Zo bleek het nuttig om CA-125 te bepalen bij patiënten met eierstokkanker, omdat patiënten met een pre-operatief verhoogd CA-125 in bloed vaak gemetastaseerde eierstokkanker hadden en daardoor een slechte prognose. Daarbij was CA-125 van waarde gedurende de behandeling en follow-up van patiënten met eierstokkanker tijdens en na aanvullende behandeling: stijging van CA-125 in bloed gedurende de behandeling danwel na de behandeling duidde op respectievelijk inadequate (chemo)therapie en een recidief. De waarde van CA-125 bij het sereus carcinoom van de uterus is echter nooit onderzocht.

Uit deze studie blijkt dat patiënten met sereus carcinoom van de uterus met gemetastaseerde ziekte, een significant hoger pre-operatief CA-125 hebben (mediaan 124.0 U/ml) in vergelijking met patiënten met dit subtype baarmoederkanker zonder uitzaaiingen (mediaan 17.5 U/ml). Een pre-operatieve waarde met een cut-off van CA-125 > 45 U/mL in het bloed is significant geassocieerd met de aanwezigheid van gemetastaseerde ziekte: patiënten met een pre-operatief CA-125 > 45 U/mL hebben een 6.3 keer zo hoog risico op het hebben van gemetastaseerde ziekte. Verder blijkt, dat naast het stadium (de uitgebreidheid) van de ziekte, een pre-operatieve waarde van CA-125 > 45 U/ml significant geassocieerd is met een slechtere overleving. Dit onderzoek toont aan dat een pre-operatief verhoogd CA-125 in bloed van een patiënte met sereus carcinoom van de uterus een goede voorspeller is voor de aanwezigheid van gemetastaseerde ziekte, en dat CA-125 een onafhankelijk risicofactor is met betrekking tot overleving. Het routinematig pre-operatief bepalen van CA-125 in bloed wordt dan ook aanbevolen in patiënten met sereus carcinoom van de uterus.
Notabene: CA-125 kan niet worden gebruikt om dit subtype van baarmoederkanker op te sporen (screening), omdat niet alle kankers het CA-125 produceren (lage sensitiviteit) en omdat er ook andere aandoeningen zijn waarbij CA-125 verhoogd is (lage specificiteit).

In **Hoofdstuk 4** wordt de frequentie van sterk afwijkende cellen dan wel kankercellen specifiek komend vanuit de baarmoeder in uitstrijkjes van de baarmoederhals onderzocht. Dit wordt vergeleken in patiënten met baarmoederkanker van het agressieve sereuze subtype versus patiënten met het prognostisch gunstigere endometrioïde subtype. Tevens wordt bekeken of uitstrijkjes van de baarmoederhals kunnen worden gebruikt als voorspeller van de prognose bij baarmoederkanker patiënten.
In Nederland werd in de jaren 70 van de 20e eeuw begonnen met een grootschalig bevolkingsonderzoek met behulp van een uitstrijkje ter preventie van baarmoederhalskanker (cervixcarcinoom). Het uitstrijkje is een simpele, doeltreffende en relatief goedkope manier om voorstadia van baarmoederhalskanker te detecteren. Het uitstrijkje kon niet worden gebruikt om vrouwen te screenen op baarmoederkanker, omdat de sensitiviteit van de uitstrijk hiervoor te laag lag. Echter, wanneer per toeval sterk afwijkende en daarom verdachte cellen, specifiek afkomstig vanuit de baarmoeder konden worden aangetoond in het uitstrijkje (abnormale uitstrijk), dan was dit een belangrijke voorspeller voor een ziekteproces in de baarmoeder. Bezien over de gehele populatie patiënten met baarmoederkanker wordt de frequentie van abnormale uitstrijkjes geschat op 25-35%.
Opvallend genoeg blijkt uit deze studie dat patiënten met het sereus carcinoom van de uterus in maar liefst 87.5% een abnormale uitstrijk hebben, specifiek duidend op een ziekteproces in de baarmoeder. Dit vergeleken met 37.8% abnormale uitstrijkjes bij patiënten met het endometrioïde carcinoom van de uterus. Verder blijkt dat een abnormale uitstrijk bij patiënten met een sereus carcinoom van de uterus geassocieerd is met de aanwezigheid van gemetastaseerde ziekte. In patiënten met het endometrioïde carcinoom van de uterus is een abnormale uitstrijk geassocieerd met baarmoederkanker zich uitbreidend tot de baarmoederhals. Echter, in beide populaties

patiënten (sereus en endometrioïde carcinoom van de uterus) wordt geen associatie gevonden tussen een abnormale uitstrijk en uiteindelijke overleving. Concluderend blijkt een abnormale uitstrijk geassocieerd met slechte prognostische factoren. Echter een abnormale uitstrijk, specifiek duidend op een ziekteproces in de baarmoeder, heeft geen prognostische waarde met betrekking tot overleving bij deze patiënten.

In **Hoofdstuk 5** wordt onderzocht wat de invloed is van het percentage sereuze histologie binnen de gehele tumor in de baarmoeder op het klinische gedrag van de kanker, op de prognose voor de patiënte en op de kans op recidief. Hiervoor worden patiënten met baarmoederkanker geheel bestaand uit het sereuze subtype vergeleken met patiënten met baarmoederkanker bestaand uit gemixte subtypes (sereus met ander histologische subtype). Tevens wordt het baarmoederslijmvlies naast de kanker uitgebreid geanalyseerd om het voorloperstadium te detecteren en beter in kaart te brengen.
Naast het feit dat het sereus carcinoom van de uterus een uitermate agressieve variant is van baarmoederkanker, is een ander bijzonder feit dat dit subtype baarmoederkanker vaak ook gemixt voorkomt met andere histologische subtypes. Met andere woorden, de patholoog-anatoom kan bij zijn microscopisch onderzoek specifiek twee (of soms zelfs drie) verschillende histologische subtypes van baarmoederkanker onderscheiden binnen het chirurgische preparaat. Ondanks dat het sereus carcinoom van de uterus mogelijk vaak gemixt voorkomt met bijvoorbeeld het prognostisch gunstiger geachte endometrioïde subtype, werd tot nu toe aangenomen dat dit gemixte sereuze type baarmoederkanker een even slechte prognose kent als het pure sereuze carcinoom, en dus even agressief zou moeten worden behandeld.
Daarnaast bestaat er nog veel onduidelijkheid en onbekendheid omtrent het voorloperstadium van het sereus carcinoom van de uterus. Voor het prognostisch gunstigere en meer voorkomende endometrioïde subtype baarmoederkanker is wel een voorloperstadium beschreven: complexe hyperplasie met atypie (CHA). Langdurige blootstelling van het baarmoederslijmvlies aan het vrouwelijke geslachtshormoon oestrogeen kan leiden tot veranderingen in dit slijmvlies, tot bovenmatige vermeerdering van dit slijmvlies (hyperplasie) en uiteindelijk in abnormale weefselaangroei, CHA genoemd. Afhankelijk van de ernst van veranderingen in het voorloperstadium CHA hebben deze patiënten 20-40% kans op het ontwikkelen van baarmoederkanker. Minder is bekend omtrent de ontstaanswijze van het sereus carcinoom van de uterus. Slechts in een aantal kleine studies is een voorloperstadium beschreven, 'endometrial intraepithelial carcinoma' (EIC) genaamd. Het werd zelden in preparaten van baarmoederkanker met een ander histologisch subtype aangetoond. Het EIC zou ontstaan in een achtergrond van één cellaag dik (atrofisch) baarmoederslijmvlies en is per definitie non-invasief. Complexe hyperplasie met atypie (CHA) werd niet of nauwelijks gevonden rondom EIC en het sereus carcinoom van de uterus. Echter, er is nauwelijks iets bekend over de rol van EIC als voorloperstadium bij gemixte vergeleken met pure sereuze carcinomen van de uterus.

Uit deze studie blijkt dat het in 46.3% van alle gevallen van een sereus carcinoom van de uterus gaat om het pure sereuze type en in 53.7% om gemixte sereuze carcinomen. Binnen de groep gemixte sereuze carcinomen van de uterus betreft het in 77.6% een combinatie met het endometrioïde histologische subtype. Verder blijkt dat, behalve patiënten met een laag stadium (geen metastasen) van de ziekte, ook patiënten met een gemixt sereus carcinoom van de uterus een betere overlevingskans hebben en een lager risico op recidief. Patiënten met puur sereuze histologie hebben een 2.9x hogere kans op recidief en een 2.6x hoger risico op overlijden vergeleken met patiënten met een gemixt sereus carcinoom van de uterus. Het voorloperstadium EIC wordt aangetroffen in 84% van alle gevallen, zonder significant verschil in incidentie tussen de groep patiënten met puur versus gemixt sereus carcinoom van de uterus. Atrofisch baarmoederslijmvlies wordt aangetroffen in 90.7% van patiënten met een puur sereus carcinoom van de uterus, terwijl CHA wordt aangetroffen in 34.7% van alle patiënten met een gemixt sereus carcinoom (p = 0.004). Concluderend blijkt dat puur sereuze histologie van de baarmoederkanker samen met een vergevorderd stadium van de ziekte de belangrijkste risicofactoren bij patiënten met sereus carcinoom van de uterus zijn voor wat betreft de kans op recidief en uiteindelijke overleving. Verder wordt EIC met een zelfde frequentie aangetroffen in zowel pure als gemixte sereuze carcinomen, suggestief voor zijn rol als voorloperstadium in beide varianten van sereus carcinoom van de uterus. De rol van CHA bij gemixt sereus carcinoom van de uterus blijft echter nog onduidelijk.
Zoals reeds eerder vermeld is het sereus carcinoom van de uterus histologisch en in klinisch gedrag identiek aan het sereus carcinoom van het ovarium (eierstok). Eierstokkanker is één van de meest sluipende en meest agressieve vorm van kanker. Er zijn nauwelijks voortekenen en als het wordt ontdekt is het bijna altijd al te laat. Vandaar dat eierstokkanker ook wel de "silent lady killer" wordt genoemd. Het sereus carcinoom van het ovarium is het meest voorkomende subtype (70-80%) van eierstokkanker. Patiënten presenteren zich ten tijde van diagnose in 60-80% van de gevallen met gemetastaseerde ziekte en hebben een slechte prognose. Jaarlijks krijgen ongeveer 1100 vrouwen in Nederland deze ziekte van wie er 1000 overlijden. Het ontdekken en diagnosticeren van eierstokkanker in een vroeg stadium, of zelfs van het voorloperstadium, zou de behandeling en prognose van deze groep patiënten beduidend kunnen verbeteren. Helaas is gebleken dat screening naar eierstokkanker met behulp van echografie, de tumormerkstof CA-125, of met andere biomarkers niet sensitief en effectief is.
De huidige gedachte omtrent de ontstaanswijze van eierstokkanker werd door de Egyptische gynaecoloog Fathalla reeds in 1971 gepostuleerd en was met name gebaseerd op epidemiologische feiten. Zo bleek een reductie in het aantal eisprongen (ovulaties) gedurende het leven van de vrouw (veroorzaakt door multipele zwangerschappen, het geven van borstvoeding, en het gebruik van orale anticonceptiva) te leiden tot een verlaging van het risico op het ontwikkelen van eierstokkanker gedurende het verdere leven. Er werd gedacht dat toenemende schade aan de eierstok, die optreedt na elke maandelijkse ovulatie, een factor is in het uiteindelijk ontaarden in kanker van de eierstok.

Echter, het type cel waaruit de eierstok bestaat lijkt helemaal niet op het sereuze subtype kankercel zoals die wordt aangetroffen in eierstokkanker; dit type cel lijkt meer op de cellen van Müller, die zich vroeg in de embryonale fase ontwikkelen tot eileiders, baarmoeder en bovenste 1/3 van de vagina. Daarnaast is tot op heden nooit een voorloperstadium van eierstokkanker in de eierstokken zelf ontdekt.

In **Hoofdstuk 6** wordt de incidentie onderzocht van een potentieel voorloperstadium van eierstokkanker in de eileiders (tubae). Hiervoor wordt een groep patiënten met een bewezen BRCA1/2 gen-mutatie waarvoor preventieve verwijdering van eierstokken en eileiders, vergeleken met een groep patiënten zonder erfelijke aanleg voor het ontwikkelen van eierstokkanker, waarbij om niet-kanker gerelateerde redenen de eierstokken en eileiders zijn verwijderd.
In de jaren '90 van de vorige eeuw werden twee genen, BRCA 1 en BRCA 2, geïdentificeerd die geassocieerd waren met een sterk verhoogde kans op het krijgen van borst- en eierstokkanker wanneer deze genen een DNA-verandering (mutatie) bevatten. Afhankelijk van welk van de twee BRCA genen gemuteerd is, is het risico op het krijgen van eierstokkanker 5-60%. Aangezien screening voor eierstokkanker tot nu toe niet effectief is gebleken wordt de meeste BRCA mutatiedraagsters geadviseerd rond hun 40ste levensjaar en na vervulling van hun kinderwens preventief de eierstokken met eileiders te laten verwijderen. Dit leidt tot een afname van het risico op kanker tot naar het gemiddelde bevolkingsrisico.
Deze reductie in risico op eierstokkanker heeft ertoe geleid, gecombineerd met het feit dat nooit een voorloperstadium werd aangetroffen in de eierstok zelf, dat onderzoekers de laatste jaren nauwkeurig de eileiders van BRCA mutatiedraagsters zijn gaan analyseren en bestuderen. Recentelijk hebben een aantal studies een mogelijk voorloperstadium voor sereus ovariumcarcinoom beschreven in de eileiders (tubae), 'serous tubal intraepithelial carcinoma' (STIC) genaamd. Vanuit embryologisch perspectief gezien stammen cellen van de eileider af van de cellen van Müller, en ontaarding van deze cellen zou leiden tot een kankercel subtype zoals dat van het sereuze ovarium carcinoom. Aangenomen wordt dat voorloper kankercellen vanuit STIC loslaten en zich hierna hechten op de eierstok en/of elders in de buik (gemetastaseerde ziekte). Echter, de incidentie van STIC als voorloperstadium varieert sterk, tussen 0.6%-8%, en de reproduceerbaarheid van de diagnose STIC is matig tot slecht. Daarbij is nog nauwelijks iets bekend over de incidentie van STIC als voorloperstadium bij patiënten zonder BRCA mutatie en dus zonder erfelijke aanleg voor eierstokkanker.
Voor deze studie worden 226 BRCA1/2 mutatiedraagsters en 105 vrouwen waar voor niet-kanker gerelateerde redenen de eierstokken en eileiders zijn verwijderd (controle groep) onderzocht. In de groep BRCA mutatiedraagsters wordt in 6% een STIC als voorloperstadium gevonden en bij 1% per toeval daadwerkelijk een vroeg stadium eierstokkanker, in tegenstelling tot de controle groep waar geen STIC of invasieve kanker wordt aangetroffen (p = 0.004). In veruit de meeste gevallen wordt het voorloperstadium STIC geïdentificeerd in het uiteinde van de eileiders (64.3%), dus in de

nabijheid van de eierstok. Een gevonden afwijking (STIC dan wel kanker) blijkt geassocieerd met een hogere leeftijd van desbetreffende vrouw. Concluderend wordt in ongeveer 6% van de BRCA mutatiedraagsters een voorloperstadium van eierstokkanker gevonden, terwijl in de eileiders van de controle groep geen afwijkingen worden gevonden. STIC lijkt dus een uniek voorloperstadium voor het sereus ovarium carcinoom al wordt het in de minderheid van de gevallen geïdentificeerd.

In **Hoofdstuk 7** wordt een alternatieve hypothese onderzocht waarbij in patiënten met eierstokkanker mogelijk een voorloperstadium aanwezig is in het baarmoederslijmvlies. Hiervoor wordt een groep patiënten met sereus ovarium carcinoom geselecteerd en van deze patiënten wordt het baarmoederslijmvlies uitgebreid geanalyseerd, op zoek naar een mogelijk voorloperstadium.
Zoals reeds eerder vermeld is tot op heden nooit een voorloperstadium van eierstokkanker geïdentificeerd in de ovaria. Hoewel recentelijk steeds meer data in de richting wijzen van STIC in de tubae als mogelijk voorloperstadium van het sereuze subtype eierstokkanker, wordt in minder dan 10% van alle BRCA mutatiedraagsters dit voorloperstadium daadwerkelijk gevonden. Interessant feit is dat vrouwen die zijn gesteriliseerd of op jonge leeftijd hun baarmoeder operatief hebben laten verwijderen om niet-kanker gerelateerde redenen, een lager risico hebben op het ontwikkelen van eierstokkanker gedurende het verdere leven. Deze afname in het risico op het krijgen van eierstokkanker is zelfs 20-60%. Daarbij moet in ogenschouw worden genomen dat bij beide ingrepen zowel de ovaria als de tubae nog steeds in het lichaam van de vrouw aanwezig zijn. Wellicht een bijzonder feit wanneer men aanneemt dat een mogelijk voorloperstadium van eierstokkanker zich in de ovaria dan wel tubae zou bevinden.
'Endometrial intraepithelial carcinoma' (EIC) is het voorloperstadium van het sereuze carcinoom van de uterus en kan in 80-90% van de gevallen met dit subtype baarmoederkanker worden geïdentificeerd. EIC is per definitie non-invasief en ontstaat in een achtergrond van atrofisch baarmoederslijmvlies. Echter van EIC is beschreven dat het ook kan voorkomen op de ovaria, in de tubae, of elders in de buikholte, zonder dat hier verder sprake is van kanker. De hypothese in dit hoofdstuk is dat voorlopercellen, afkomstig van het EIC, loslaten en migreren via de eileiders richting eierstok en buikholte. Dit fysiologische migratie proces wordt bijvoorbeeld ook gezien tijdens een normale menstruatie van een vrouw, waarbij bloed en delen baarmoederslijmvlies behalve richting vagina uitgang ook via de eileiders richting buikholte bewegen. De kleine hoeveelheden bloed die daardoor in de buikholte terecht komen prikkelen het buikvlies en kunnen de typische menstruatiepijn in de (onder)buik geven. Ook worden bijvoorbeeld na gemeenschap de zaadcellen vanuit de baarmoeder naar de eileiders gedirigeerd.
Voor dit onderzoek wordt een groep patiënten met sereus ovarium carcinoom geselecteerd. Van deze patiënten wordt het baarmoederslijmvlies en eileiders uitgebreid geanalyseerd. In 9 van de 38 gevallen wordt naast de eierstokkanker ook een mogelijk voorloperstadium in het baarmoederslijmvlies van deze patiënten geïdentificeerd, het EIC. In deze groep patiënten wordt geen STIC geïdentificeerd. Om een daadwerkelijke relatie te kunnen aantonen tussen de

eierstokkanker en het mogelijke voorloperstadium, wordt het eiwitprofiel onderling vergeleken en het DNA van beide afwijkingen geanalyseerd. Het blijkt dat voor vier verschillende eiwitten het profiel nagenoeg identiek is voor zowel het sereuze carcinoom van het ovarium als het EIC. Verder wordt in 33% van de gevallen een unieke en identieke DNA-mutatie aangetoond in beide afwijkingen. Tot slot blijkt dat de cellen van de eierstokkanker op DNA niveau sterker afwijkend zijn ($p = 0.039$), met sterk veranderde cellulaire processen ($p = 0.039$), vergeleken met cellen van EIC. Dit laatste is onwaarschijnlijk wanneer EIC geen voorloperstadium maar een metastase zou zijn van de eierstokkanker. Concluderend ondersteunen deze data voor het eerst de hypothese dat EIC in het baarmoederslijmvlies een mogelijk voorloperstadium is van het sereuze carcinoom van het ovarium. Met deze mogelijke oorsprong in de baarmoeder is ook het beschermende effect van sterilisatie dan wel operatieve verwijdering van de baarmoeder op het krijgen van eierstokkanker te verklaren. Ook hebben multipele zwangerschappen, het geven van borstvoeding, en het gebruik van orale anticonceptiva (allen factoren geassocieerd met een verlaging van het risico op het ontwikkelen van eierstokkanker) duidelijk een invloed op het baarmoederslijmvlies en dus mogelijk op het ontstaan van EIC in het slijmvlies.

In **Hoofdstuk 8** worden de belangrijkste bevindingen van dit proefschrift samengevat en bediscussieerd. Met name het belang van het opzetten van een goede prospectieve klinische studie naar de juiste behandeling van het sereuze carcinoom van de uterus wordt hier onderstreept. In afwachting van een dergelijke studie wordt een algoritme geboden die kan helpen in de klinische besluitvorming. Verder worden de moeilijkheden en valkuilen voor zowel de gynaecoloog als de patholoog-anatoom met betrekking tot het sereuze carcinoom van de uterus bediscussieerd. Ook worden de rollen van EIC en STIC als mogelijke voorloperstadia van eierstokkanker besproken. Tot slot worden enige ideeën voor toekomstig onderzoek aangedragen. Zo wordt gesuggereerd dat een toekomstige prospectieve studie een studie zou kunnen zijn waarin BRCA mutatiedraagsters, met een verhoogd risico hebben op het krijgen van eierstokkanker, conventioneel worden behandeld door chirurgisch zowel eierstokken als eileiders te verwijderen, dan wel slechts alleen de eileiders te verwijderen. Hoewel controversieel, een dergelijke studie zou voor het eerst daadwerkelijk kunnen bewijzen dat eierstokkanker niet vanuit de eierstok zelf ontstaat.
Uiteindelijk is de gedachte dat het sereuze subtype ovarium carcinoom mogelijk niet ontstaat vanuit de eierstok, maar een secundaire vorm van kanker is met een oorsprong in de eileiders dan wel het baarmoederslijmvlies.

Appendices

Dankwoord, Curriculum Vitae en Bibliography

DANKWOORD

Het is dan zover: het boekje is af!! Hiermee is een eind gekomen aan één van de meest hectische, maar tegelijkertijd meest bijzondere en leerzame periodes in mijn leven. Dit was natuurlijk nooit gelukt zonder de hulp, steun en het vertrouwen van vele mensen om mij heen. Bedankt daarvoor! Een aantal mensen wil ik hieronder nog even speciaal benoemen.

Als eerste natuurlijk mijn "promotie-team":
Mijn promotor Prof. Dr. Massuger, beste Leon, wij hadden inmiddels alweer 5 jaar geleden eigenlijk maar 2 gesprekken op een vrijdagmiddag nodig om te weten wat we allebei wilden, wat de bedoeling ging zijn, en hoe we het wilden aanpakken. Ik heb het wel eens "liefde op het eerste gezicht" genoemd, op het professionele vlak dan… ☺ Jouw kracht om mensen te motiveren en te enthousiasmeren is erg bijzonder, en samen met een hoop intelligentie, mensenkennis, en de continue stroom aan ideeën maakt jou voor mij een hele goede en fijne promotor. Ik, door jou wel eens 'jonge hond' genoemd, heb veel van je mogen leren en niet alleen omtrent mijn eigen onderzoek. Ik heb onze gesprekken op de vrijdagmiddag/avond en in de weekenden altijd zeer gewaardeerd. Ohw, nog 1 ding: uiteraard wil ik nog steeds revanche voor die verloren pot squash!
Mijn co-promotor, Dr. van Ham, beste Maaike, je kwam er iets later bij, maar je pakte het meteen goed op en wierp jezelf op als een soort beschermengel van mij. Jij hield een oogje in het zeil of ik niet teveel werkte, ik het allemaal wel trok, sociaal nog wel aan dingen toekwam, en je was er voor me op persoonlijk vlak wanneer het wel eens even wat minder met me ging. Het laagdrempelige en persoonlijke contact heb ik altijd gewaardeerd! Je bent een echte mensen-dokter.
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In aansluiting hierop wil ik ook de pathologen S. Zomer (CWZ), A. Wiersma van Tilburg (Rijnstate) en M. Bol (UMC) benoemen. Beste Saskia, Anne, en Mijke, jullie hebben een monsterklus geleverd door alle coupes individueel te reviseren. Dat maakte onze database en onze pathologische bevindingen uiteindelijk ook zo sterk. Gelukkig werd dat recent ook beloond met goede publicaties. Ik denk dat we hier trots op mogen zijn!

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en samenwerkingen werden aangegaan. Met name Dr. van der Laak, beste Jeroen, wil ik bedanken voor zijn hulp met hoofdstuk 7, ongelooflijk hoe jij microscopen en software kunt gebruiken om de meest uiteenlopende zaken te automatiseren en te berekenen. Speciaal moet ik hier nog noemen Dr. van Kempen, beste Léon, jij hebt samen met mij in kort tijdsbestek volle bak gewerkt aan de data die hebben geleid tot hoofdstuk 7 van dit proefschrift. Doch controversieel zijn we erg trots op de bevindingen en zonder jouw visie en hulp was het me niet gelukt. Gezien jouw intelligentie, werklust, inzicht en open denk wereld verbaasde het me natuurlijk niets dat je naar Montreal, Canada, werd gehaald. Je hebt die goede positie verdiend. Drink daar maar een sinas op! ;)

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CURRICULUM VITAE

Thijs Roelofsen werd geboren op 30 November 1981 te Lichtenvoorde, waarna hij zijn jeugd doorbracht in Zieuwent. In 2001 behaalde hij zijn VWO-diploma aan het R.K. Scholengemeenschap Marianum te Groenlo. Nadat hij in verkorte tijd aan de HLO te Enschede in 2 verschillende richtingen afstudeerde (Medische Biochemie en Medische Biotechnologie), verliet hij de Achterhoek om vanaf 2004 Medische Biologie te gaan studeren aan de Radboud Universiteit te Nijmegen. In zijn Master-fase deed hij onderzoek bij de afdeling Research & Development van het farmaceutische bedrijf Organon te Oss (thans MSD) onder leiding van dr. Koen Dechering, dr. Roel Arends en dr. Jan Gossen. Hij studeerde af binnen de afdeling Tumor Immunologie (TIL) van het NCMLS te Nijmegen, onder leiding van dr. Stefan Nierkens en prof. dr. Gosse Adema. Beide stages leidden tot een wetenschappelijk publicatie. Ondanks het aanbod van een baan als fulltime promovendus binnen het TIL besloot hij vanaf 2007 geneeskunde te gaan studeren, met name omdat hij in het onderzoek het contact met patiënten altijd erg heeft gemist. Nog immer enthousiast voor wetenschappelijk onderzoek kwam hij in gesprek met prof. dr. Leon Massuger, en werd naast zijn studie geneeskunde begin 2008 ook gestart met een promotieonderzoek bij de pijler Gynaecologische Oncologie van het UMC St. Radboud (promotor prof. dr. Leon Massuger en copromotores dr. Maaike van Ham en dr. Hans Bulten). Hierbij werd gewerkt aan de studies die uiteindelijk hebben geleid tot het proefschrift dat nu voor U ligt. Na zijn Bachelor geneeskunde in 2009 werkte hij ruim 1 jaar fulltime aan zijn promotieonderzoek, om in 2010 te starten met zijn coschappen en daarnaast parttime zijn promotieonderzoek af te ronden. Het coschap Obstetrie & Gynaecologie in het Rijnstate ziekenhuis te Arnhem in 2011 bevestigde zijn interesse en voorliefde voor dit specialisme, en van September t/m November 2012 liep hij met veel enthousiasme zijn senior coschap Obstetrie & Gynaecologie in het Jeroen Bosch ziekenhuis te Den Bosch. In Januari 2013 behaalde hij zijn arts-examen en is nu met veel plezier werkzaam als arts-assistent Gynaecologie (ANIOS) in het Rijnstate ziekenhuis te Arnhem.

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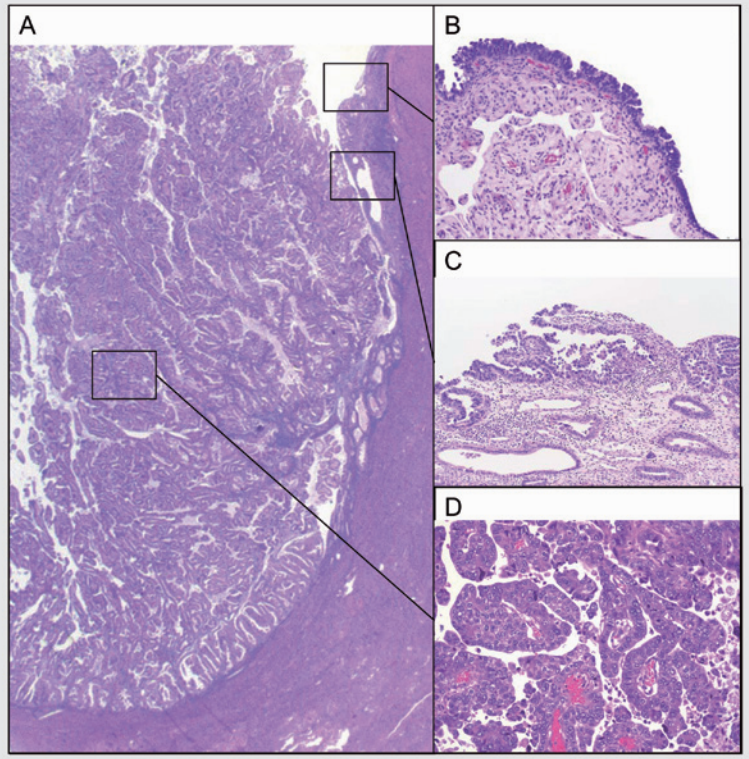
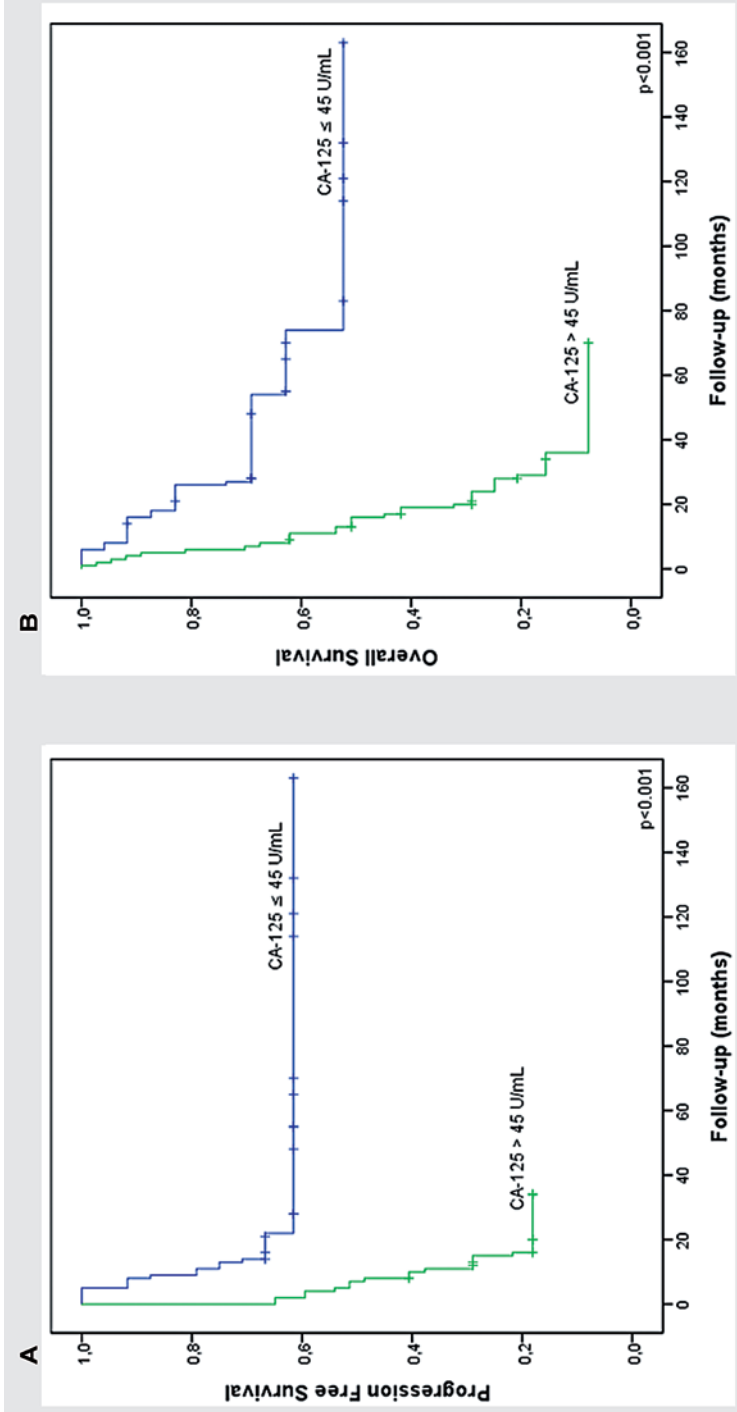
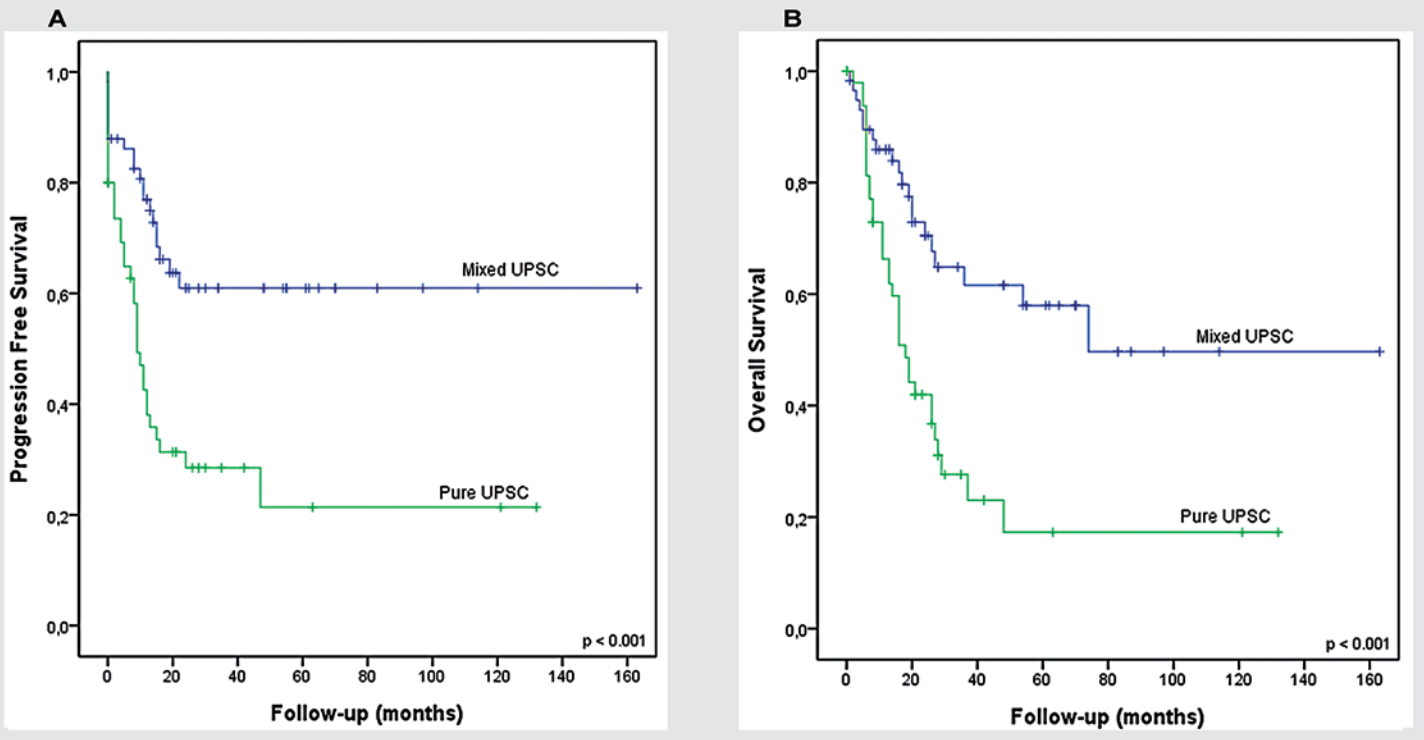
Colour Appendix



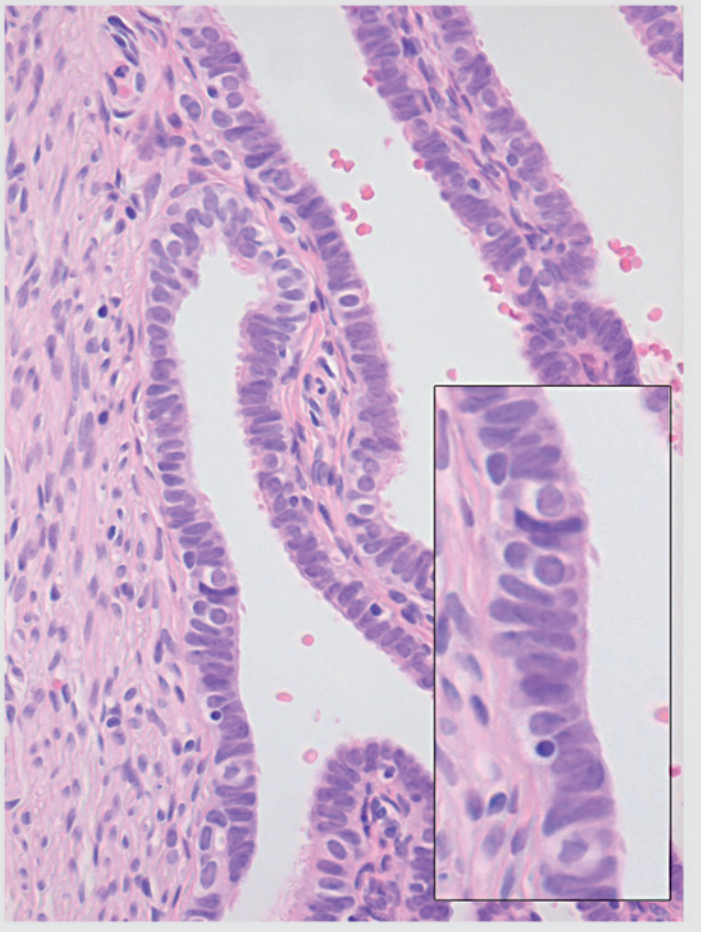
Chapter 1, figure 1: Uterine papillary serous carcinoma (UPSC). **A)** Overview of full-blown UPSC with invasive properties and complex papillary growth (level of magnification: 50x). **B & C)** The distinctive precursor lesion endometrial intraepithelial carcinoma (EIC) represents malignant transformation of atrophic surface endometrium without myometrial invasion, and is immediately adjacent to the invasive UPSC (level of magnification: 200x). **D)** In detail view of UPSC, with its complex papillary growth, numerous mitotic figures, and marked nuclear atypia (level of magnification: 400x).



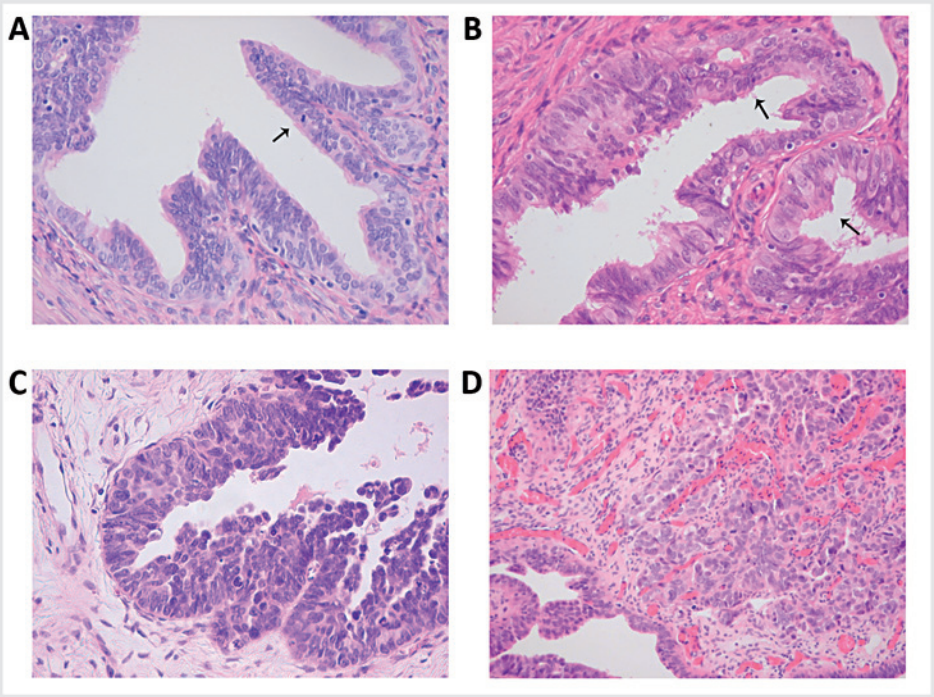
Chapter 3, figure 1: Kaplan-Meier estimates of **A)** Progression Free Survival (PFS) and **B)** Overall Survival (OS) of uterine papillary serous carcinoma patients with preoperative serum CA-125 ≤ 45 U/mL (*N* = 24, upper line) and patients with serum CA-125 > 45 U/mL (*N* = 37, lower line) ($p < 0.001$, for both PFS and OS). Vertical bars indicate patients with censored data.



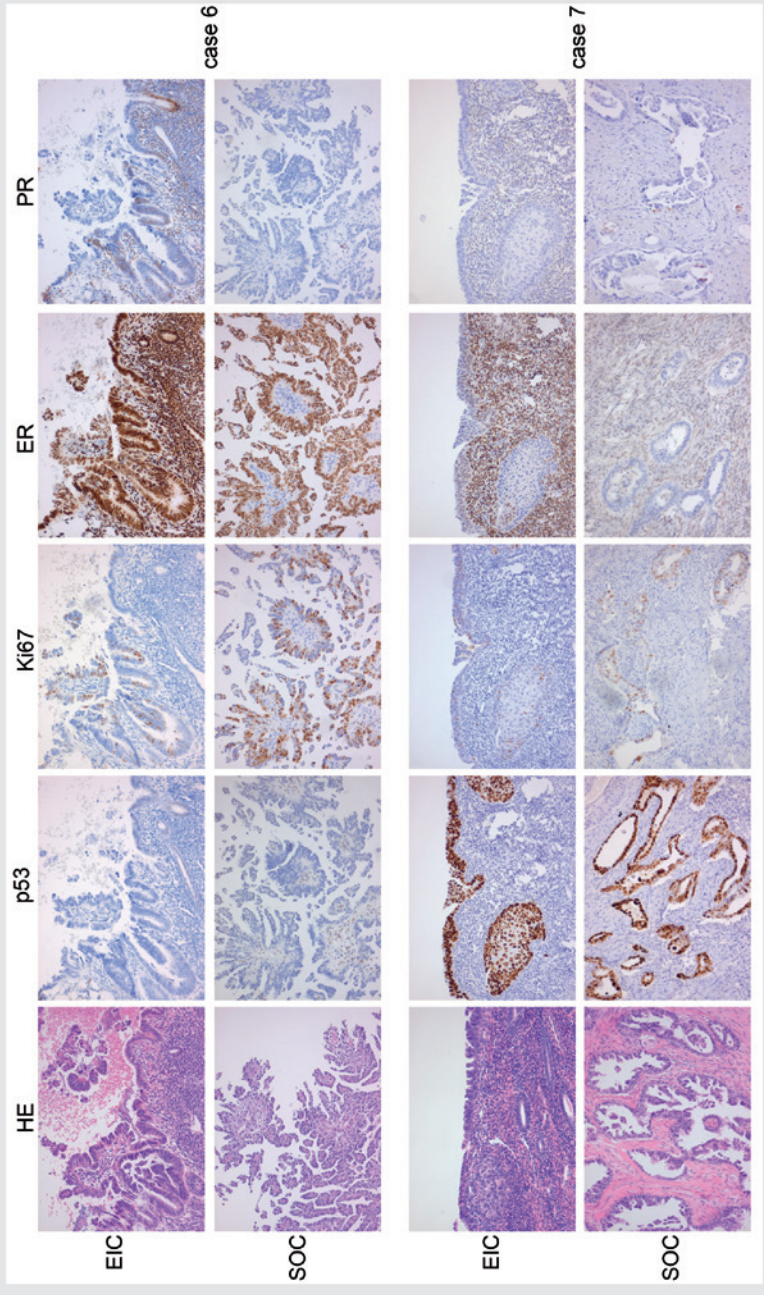
Chapter 5, figure 1: Kaplan-Meier estimates of **A)** progression free survival and **B)** overall survival of patients with uterine papillary serous carcinoma, showing pure uterine papillary serous carcinoma histology (100% serous component; $N = 50$, lower line) and patients with mixed UPSC histology (10–90% serous component; $N = 58$, upper line). Log-rank test $p < 0.001$ for both panels. Vertical bars indicate patients with censored data.



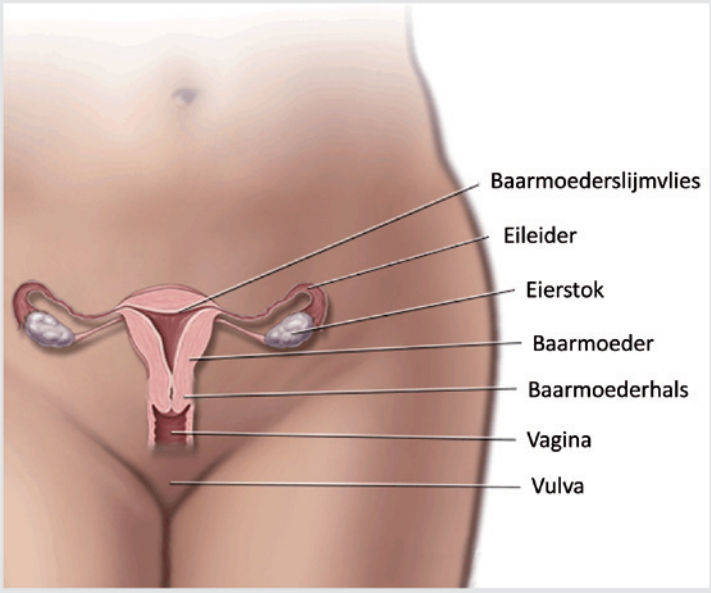
Chapter 6, figure 1: Benign fallopian tube epithelium with secretory and ciliated cells (level of magnification: 200×; level of magnification of inset: 400×).



Chapter 6. figure 2: Tubal epithelial lesions (level of magnification: 200×). **A)** Fallopian tube epithelium with hyperplasia, showing cellular crowding, but without atypical nuclei (arrow: mitotic figure). **B)** Minor epithelial atypia in the fallopian tube showing cellular crowding, slight cellular atypia and nuclei with small nucleoli, but without loss of polarity of the epithelial cells (arrows). **C)** Tubal intraepithelial carcinoma (STIC) with cellular crowding, stratification of nuclei, loss of polarity and severe atypia of the nuclei. **D)** Fallopian tube carcinoma, including overlying atypical epithelium.



Chapter 7, figure 1: Representative sections of endometrial intraepithelial carcinoma (EIC) and serous ovarian carcinoma (SOC) stained for different proteins by immunohistochemistry. Case numbers correspond to the cases summarized in Tables 1 & 2. Abbreviations: HE, hematoxylin-eosin staining; ER, estrogen receptor; PR, progesterone receptor.



Chapter 9, figuur 1: het vrouwelijke voortplantingsstelsel.